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PESTICIDE RESIDUE ANALYSIS IN WATER
TRAINING MANUAL

ENVIRONMENTAL PROTECTION AGENCY

SEPTEMBER 1974

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PESTICIDE RESIDUE ANALYSIS IN WATER

This course is designed for analytical chemists and others having technical responsibility for the collection, identification and measurement of organic pesticides in the aquatic environment.

**ENVIRONMENTAL PROTECTION AGENCY
Office of Water Programs
TRAINING PROGRAM**

CONTENTS

<u>Title or Description</u>	<u>Outline Number</u>
Distribution of Pesticides in the Environment	1
Chemistry of Pesticides	2
Behavior of Pesticides in the Aquatic Environment	3
Use of Extraction Methods in Pesticide Analysis	4
Use of Extraction Methods in Pesticide Analysis - Fruits and Vegetables	5
Thin-Layer Chromatography	6
Gas Chromatography	7
Introduction to Gas-Liquid Chromatography	8
Characteristics of the Flame Ionization Detector	9
Quantitation in Gas Chromatography	10
Special Techniques Required for the Analysis of Phosphorus and Herbicidal Compounds	11
Infrared Instrumentation	12
Principles of Infrared Spectroscopy	13
Pesticide Residue Analysis of Fish by Infrared Spectrophotometry	14
Pesticide Residue Analysis in Complex Substrates by Infrared Spectrophotometry	15
Biomonitoring of Industrial Effluents	16
Methods of Detection of Cholinesterase Inhibition	17
Principles of Column Selection and Preparation; Column Packing and Conditioning	18
Trouble Shooting the Gas Chromatograph	19
Sampling for Pesticide Residue Analysis	20
Design of a Pesticide Sampling Program for the Water Environment	21

<u>Title or Description</u>	<u>Outline Number</u>
Collection and Preservation of Water, Mud, Fish, Blood, and Other Biological Specimens for Pesticide Analysis	22
Comparative Methodology and Quality Control	23
Other Methods Used in Pesticide Residue Analysis	24
Determination of Cholinesterase Inhibition in Fish Brain	25
Analysis of Water for 2, 4-D	26
Analysis of Fish and Related Biological Specimens for Organochlorine and Organophosphorus Pesticides	27
Sediment Extraction Procedure	28
Sampling for Pesticides and General Organic Analysis	29
Method for Organochlorine Pesticides in Industrial Effluents	30
Method for Organophosphorus Pesticides in Industrial Effluents	31
Method for Polychlorinated Biphenyls (PCB's) in Industrial Effluents	32
Method for Triazine Pesticides in Industrial Effluents	33
Method for O-Aryl Carbamate Pesticides in Industrial Effluents	34
Method for N-Aryl Carbamate and Urea Pesticides in Industrial Effluents	35
Method for Chlorinated Phenoxy Acid Herbicides in Industrial Effluents	36
Table 1 - Order of Elution of Standard Insecticides from Alumina Adsorption Column	
Table 2 - Physical Constants of Solvents	

DISTRIBUTION OF PESTICIDES IN THE ENVIRONMENT

I PESTICIDE USAGE AND ENVIRONMENT RESEARCH

A Usage Trends

Since the end of World War II there has been an explosive expansion in pest control by chemicals. Sales in the U.S. have increased 84% in the decade since 1955 and production in 1965 was 875 million pounds.

1 Number of compounds in use

- a Presently there are about 650 kinds of compounds in use. At least 200 of these are of first rate economic importance.

B Environmental Research

Environmental research has been concentrated on relatively few compounds. DDT has been studied the most. The chlorinated cyclodienes (aldrin, endrin, and dieldrin) have also received more than average attention.

1 Toxicological significance

- a Damage to organisms containing high residues levels has been repeatedly demonstrated. However, the significance of residues levels which are normally found in natural populations of organisms is largely unevaluated.

II PESTICIDE RESIDUES IN AIR

A Extent of Measurements

There are very few reports describing the presence and amount of pesticides in ambient air over urban or rural areas. Most measurements have been made to estimate the exposure associated with the direct use of pesticide and its potential hazard to agricultural workers.

1 Compounds and concentrations found in air and near treated orchards or forests.

- a The following compounds have been isolated from air: parathion, malathion, methyl parathion, BHC, and DDT.
- b The quantities isolated have generally been less than 0.20 mg/m^3 ; however, in forest applications, BHC levels varied from $2.6 - 12.5 \text{ mg/m}^3$ for ground applications, and from 4.1 to 53.7 mg/m^3 for aerial applications.

III RESIDUES IN MAN AND HIS DIET

A Extent of Measurements

The compound which has been studied most carefully with respect to levels in diet and storage in humans is DDT. Dieldrin and lindane have been found to be stored in people without occupational exposures. However, at present, with the exception of DDT, only qualitative information is available about dietary content and human body burdens.

B DDT in Man

1 Non-occupational exposure and residue levels related to diet.

- a The average intake of DDT in diet is estimated to be $184 \text{ } \mu\text{g/person/day}$.
- b The average body burden is about 5 ppm DDT or 12 ppm for all DDT derived compounds (i.e., DDE, DDD), expressed as DDT.
- c Eskimos and abstainers from meat have less stored DDT than the general population.

2 Occupational exposure

- a Agricultural applicators store about 3 times the level of DDT found in the general population.
- b Formulators have been found to contain as much as 600 ppm DDT or 1,000 ppm of DDT-derived material.

IV INSECTICIDE RESIDUES IN WATERSHEDS AND WATERWAYS

A Extent of Measurements

A large number of residue measurements have been made in aquatic ecosystems. However, relatively few systematic measurements have been made on a basin basis where residue inputs from terrestrial environments have been related to the quantity and duration of pollution in the aquatic environment.

B Basin Studies

1 Soil

- a Residues of DDT, DDE, BHC, and toxaphene were found in cotton field soil a year after use. Concentrations were in the mid-parts/billion to the low parts/million range.

2 Water

- a Residues of toxaphene and BHC were found in water year-round, generally in concentrations less than 0.5 ppb.

- b The occurrence of toxaphene appeared to be independent of usage in the basin, but BHC concentrations were related to insecticide use during the summer months.

- c Conventional water treatment practices failed to remove these residues.

3 Mud and suspended sediments

- a Mud and suspended sediments contained residues of DDT and DDE, generally at the same level as noted in cotton field soil.
- b Toxaphene and BHC, although present in water, was generally absent from mud and suspended sediment.

4 Bottom fauna

- a Macro aquatic invertebrates (insects, snails, etc.) contained DDT and DDE, at about the same levels noted in cotton field soil.

5 Fish

- a Fish contained residues of BHC, toxaphene, DDT, and DDE in concentrations comparable to that noted in cotton field soil.
- b Residue concentrations in the non-edible portions of the fish were from 3 - 4 times greater than those in the edible portion.

REFERENCE

Chichester, C.O. (Editor). Research in Pesticides. Academic Press, New York, London. 1965. 380 pp.

This outline was prepared by A. R. Grzenda, former Chief, Aquatic Ecology Activity, Southeast Water Laboratory, Athens, GA.

CHEMISTRY OF PESTICIDES

I INTRODUCTION

A Over 900 Chemical Species

B More than 90,000 Formulations

C Classification of Pesticides

1 Use

a Insecticides

b Herbicides

c Fungicides

d Rodenticides

2 Chemical

a Organohalides

b Organophosphates

c Carbamates

d Carbonates

e Thiocarbamates

f Inorganics

g Metallo-organics

h Substituted anilines

i Substituted ureas

j Substituted amides

k Triazines

l Triazoles

m Substituted organic acids

n Quinones

o Organosulfurs

p Substituted imides

q Phenols

r Miscellaneous organics

II STRUCTURAL CHARACTERISTICS

A Insecticides

1 Organochlorine

a Chlorobenzilate types

1) p p' DDT

2) o p DDT

3) Methoxychlor

4) TDE (DDD)

5) Perthane

6) Kelthane

b Aldrin group

1) Aldrin (HHDN)

2) Dieldrin (HEOD)

3) Endrin

4) Chlordane

5) Heptachlor

6) Heptachlor epoxide

c Benzene hexachloride

1) Lindane

2) Other isomers

d Fumigants

1) Methyl bromide

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c Benzene hexachloride

1) Lindane

2) Other isomers

d Fumigants

1) Methyl bromide

- 2) Ethylene dibromide
 - 3) Ethylene dichloride
 - 2 Organophosphate
 - a Alkene type
 - 1) Bidrin
 - 2) Dichlorovos (DDVP)
 - 3) Trichlorofon
 - b Alkane types
 - 1) Dimethoate
 - 2) Malathion
 - 3) Phorate (Thimet)
 - c Aryl type
 - 1) Parathion
 - 2) Diazinon
 - 3) Methyl parathion
 - 4) Ronnel
 - 5) Guthion
 - 6) EPN (phosphonate)
 - d Tetraethylpyrophosphate (TEPP)
 - 3 Carbamate
 - a Monomethyl
 - 1) Carbaryl (Sevin)
 - 2) Temik
 - 3) Zectran
 - b Dimethyl
 - 1) Dimetilan
 - 4 Carbonate
 - a Dessin
 - 5 Miscellaneous
 - a Inorganic
 - 1) Arsenates
 - 2) Cyanide compounds
 - b Naturally occurring
 - 1) Pyrethrum
 - 2) Sabadilla
 - c Sulfur compounds
 - d Dinitrophenols
 - e Other synthetics
- B Herbicides
- 1 Carbamate
 - a CIPC (chloroisopropylcarbamate)
 - 2 Thiocarbamate
 - a Ordram
 - 3 Urea
 - a Monuron
 - 4 Metallo-organics
 - a Methane arsonic acid
 - b Cacodylic acid (dimethyl arsenic acid)
 - 5 Inorganics
 - a Arsenic
 - b Ammonium sulfamate
 - c Sodium borates

- d Sodium chlorate
- e Sulfates
- 6 Triazoles
 - a Amino Triazole
- 7 Triazines
 - a Simazine
- 8 Phenols
 - a DNOC
- 9 Anilines
 - a Trifluralin
 - b Stamm
- 10 Substituted amides
 - a Diphenamid
- 11 Substituted organic acids
 - a Benzoic acid
 - b 2, 4-D
 - c Dacthal
 - d Dalapon
- 12 Miscellaneous organics
 - a Petroleum hydrocarbons
 - b Pyrazon
 - c Nitriles
 - d Mercaptans
- C Fungicides
 - 1 Heavy metals
 - a Inorganic and organic copper
 - b Copper oxyquinolinolate (Bioquin)
 - c Mercury - inorganic and organic
 - d Cadmium complexes
- 2 Organohalides
 - a Methyl bromide
 - b Ethylene dibromide
 - c Chloropicrin
 - d Chlorinated benzenes
 - e Parachloronitrobenzene
 - f Pentachlorophenol
- 3 Quinones
 - a Chloranil (tetrachloro-p-benzoquinone)
 - b Dichlone (2, 3-dichloro-1, 5-Naptho-quinone)
- 4 Triazines
 - a Dyrene
- 5 Elemental sulfur
- 6 Organosulfur compounds
 - a Thiram (tetramethyl thiuram disulfide)
 - b Sulfa drugs
 - 1) Sulfinates ($R-SO-R$)
 - 2) Sulfonates ($R-SO_2-R$)
- 7 Substituted imides
 - a Captan
- 8 Thiocarbamates
 - a Dialkyldithiocarbamates
 - 1) Ferbam
 - 2) Ziram

b Alkylendisithiocarbamates

- 1) Nabam
- 2) Zineb
- 3) Maneb

b Stable to light

- c Stable to heat
- d Stable to acids
- e Dechlorinated to trichlorobenzenes by alkali

III REACTIONS

A DDT

- 1 Persistent
- 2 Stable to acid
- 3 Stable to oxidation
- 4 Dehydrochlorinated by alkali and organic bases
- 5 Dehydrochlorinated at temperatures above the melting point
- 6 Biochemical modifications
 - a Dechlorinated by iron porphyrin complexes (DDD)
 - b Oxidized to 4, 4'' dichlorobenzophenone
 - c Hydrolyzed to DDA
 - d Dehydrochlorinated to DDE

B Aldrin

- 1 Stable to heat
- 2 Stable to weak acids
- 3 Stable to alkalies
- 4 Oxidized to dieldrin both in environment and by metabolism
- 5 Not persistent as aldrin

C Benzene hexachloride

- 1 Lindane
 - a Stable to oxidation

D Fumigants

1 Methyl bromide

- a Stable to heat, light, and oxidation
- b Methylates the amino acid, histidine - Br displaced
- c Stable to acids and alkalies
- d Volatile - not persistent

E Organophosphates

1 Parathion

- a Decomposes at high temperatures
- b Hydrolyzed by alkali (o/p bond)
- c Hydrolyzed by acids (H_3PO_4)
- d Stable to light
- e Oxidized to paraoxon
- f Metabolically hydrolyzed to paranitrophenol
- g Reaction with acetylcholinesterase

F Carbamates

1 Carbaryl

- a Decomposes on heating to isocyanate and corresponding alcohol
- b Alkaline hydrolysis to above products
- c Stable to acid hydrolysis

- d Conjugates on metabolism
 - e Stable to oxidation
 - f Stable to light
- G Thiocarbamates and Dithiocarbamates
- 1 Thiram
 - a Chelation
 - b Disulfide exchange
 - c Acid hydrolysis to CS_2 and the corresponding amine
 - d Stable to oxidation
 - e Stable to heat
 - f Stable to reduction
- H Metallo-organics
- 1 Methane arsonic acid
 - a Strong acid
 - b Reduced to CH_3AsO
 - c Oxidized to ortho arsenic acid
 - d Stable to heat
 - e Salt formation with alkali
 - f Hydrolyzes with water to $\text{As}(\text{OH})_3$
 - g Persistent arsenic residue
- I Substituted Anilines
- 1 Trifluralin
 - a Stable to oxidation
 - b Stable to heat
 - c Stable to acid hydrolysis
 - d Stable to alkaline hydrolysis
 - e Nitro groups reduced to amino groups
- f Modified product fairly persistent
- 2 Stamm
- a Stable to oxidation
 - b Stable to reduction
 - c Stable to heat
 - d Yields 2,3-dichloroaniline on alkaline hydrolysis
 - e Yields 3,4-dichloroaniline on acid hydrolysis
 - f Fairly persistent
- J Substituted Urea
- 1 Monuron
 - a Stable to oxidation
 - b Stable to reduction
 - c Decomposes on heating
 - d Acid hydrolyzed to p-chloroaniline
 - e Alkaline hydrolyzed to p-chloroaniline
 - f Moderately persistent
- K Substituted Amides
- 1 Diphenamid
 - a Stable to oxidation
 - b Stable to reduction
 - c Stable to heat
 - d Acid hydrolyzed to diphenyl acetic acid and secondary amine
 - e Alkaline hydrolyzed to diphenyl acetic acid
- L Triazoles
- 1 Amino triazole

- a Oxidizes to a coupled diazo product
- b Reduction is still in question
- c Stable to heat
- d Stable to acid hydrolysis
- e Stable to alkaline hydrolysis

M Triazines

1 Simazine

- a Stable to oxygen
- b Reductively dechlorinated
- c Stable to heat
- d Acid hydrolysis substitutes hydroxy for chlorine-dechlorination
- e Alkaline hydrolysis as above

N Substituted Organic Acids

1 Dalapon

- a Stable to oxidation
- b Stable to reduction
- c Stable to heat
- d Stable to acid hydrolysis
- e Dechlorinated by alkaline hydrolysis to $\text{CH}_3\text{CO COOH}$ (Method of Analysis)

2 Daethal

- a Stable to oxidation
- b Stable to reduction
- c Stable to heat
- d Dealkylated by alkaline hydrolysis

3 2, 4-Dichlorophenoxyacetic acid

- a Stable to oxidation

- b Stable to reduction
- c Stable to heat
- d Forms 2, 4-dichlorophenol in ultra violet light and reaction with halogen acids (analytical method)

O Heavy Metals

- 1 Reactions are those of any metal organic or inorganic compound
- 2 Mercury - very toxic
- 3 Atomic absorption - best analytical tool

P Other fungicides are not sufficiently toxic to be of concern

IV RELATIVE TOXICITIES OF SELECTED PESTICIDES

1 Oral LD_{50} - rats

- a Aldrin - 67 mg/kg
- b Arsenates - 1 mg/kg
- c Carbaryl - 560 mg/kg
- d 2, 4-D - 500 mg/kg
- e DDT - 108 mg/kg
- f Diazinon - 100 mg/kg
- g Dieldrin - 100 mg/kg
- h DNOC - 40 mg/kg
- i Endrin - 5 mg/kg
- j Ferbam - 4000 mg/kg
- k Heptachlor 130 mg/kg
- l Heptachlor epoxide - 65 mg/kg
- m Malathion - 2500 mg/kg
- n Guthion - 10 mg/kg

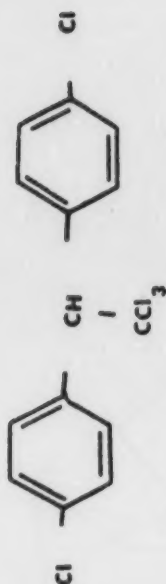
o Methoxychlor - 5000 mg/kg

p Nabam - 395 mg/kg

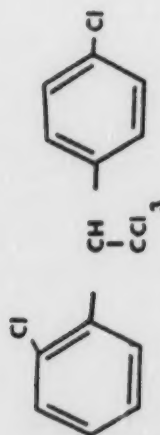
q Parathion - 4 mg/kg

r Paraoxon - 3.5 mg/kg

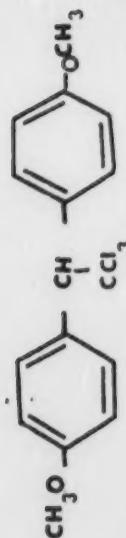
s TDE - 300 mg/kg



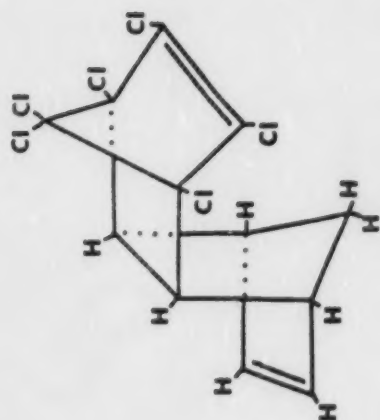
p,p' DDT



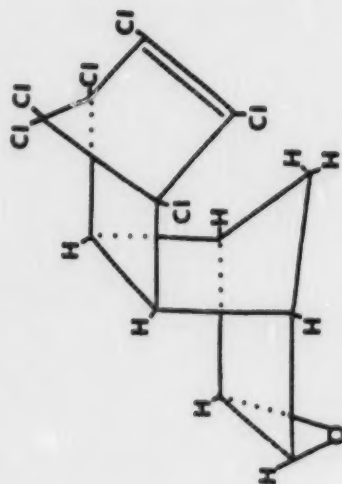
o,p' DDT



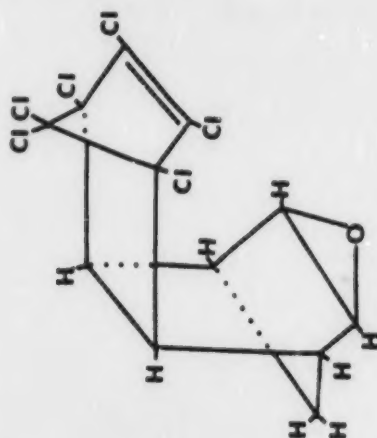
METHOXYCHLOR



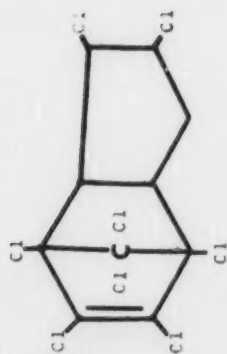
ALDRIN



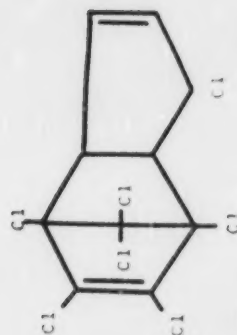
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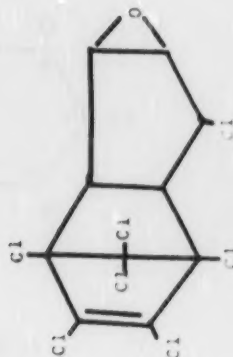
ENDRIN



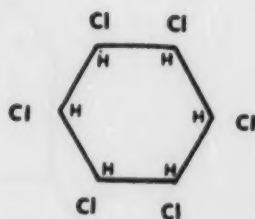
CHLORDANE



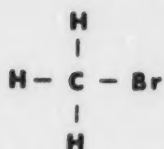
HEPTACHLOR



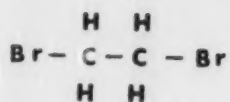
**HEPTACHLOR
EPOXIDE**



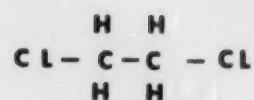
LINDANE



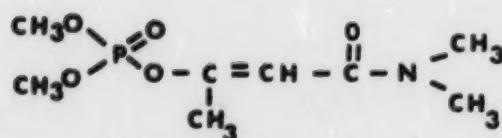
METHYL BROMIDE



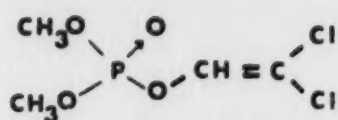
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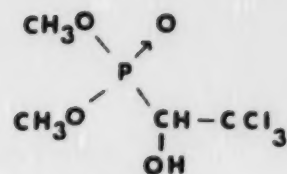
ETHYLENE DICHLORIDE



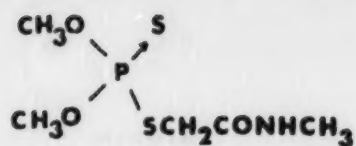
BIDRIN



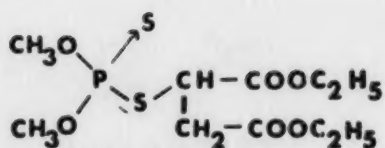
DICHLORVOX (DDVP)



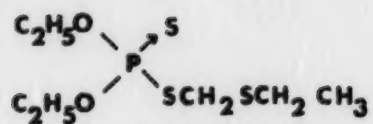
TRICHLORFON



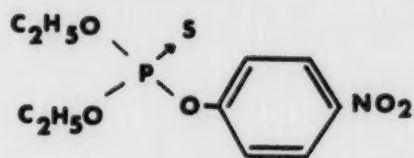
DIMETHOATE



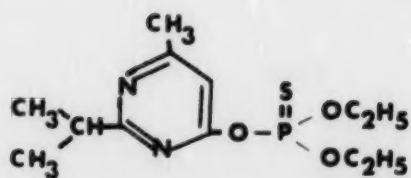
MALATHION



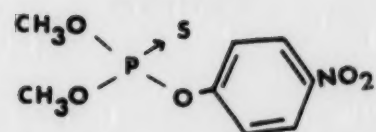
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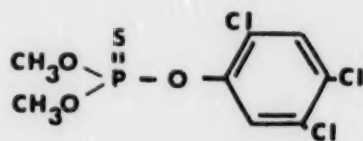
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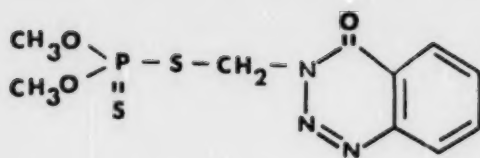
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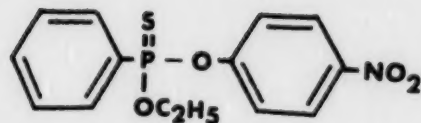
METHYL PARATHION



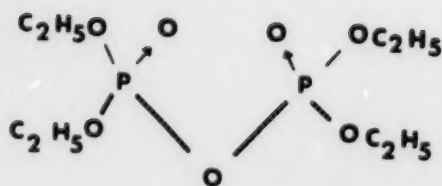
RONNEL



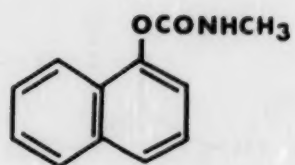
GUTHION



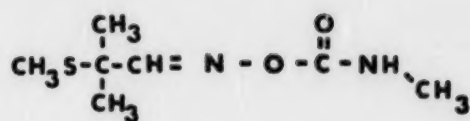
EPN



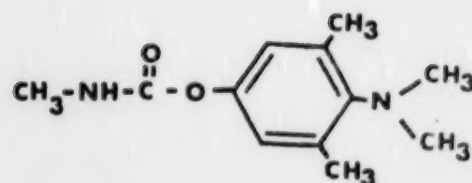
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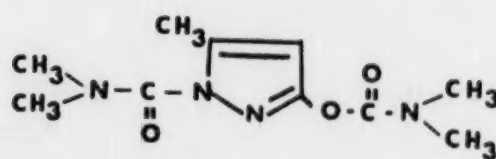
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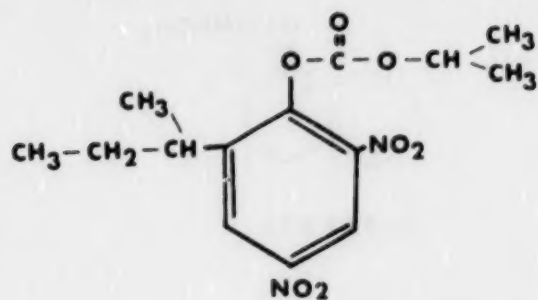
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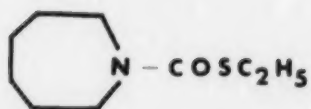
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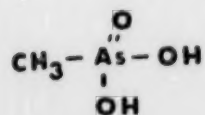
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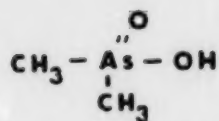
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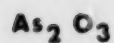
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METHANE ARSONIC ACID



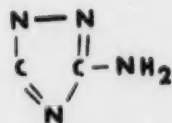
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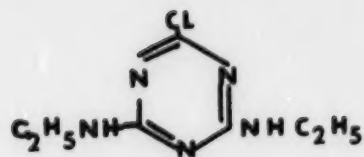
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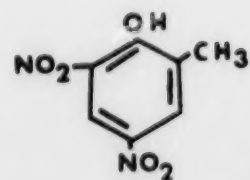
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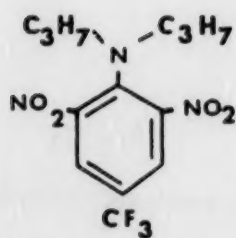
AMINO TRIAZOLE



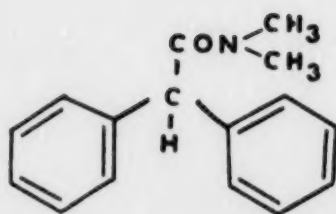
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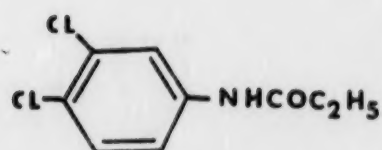
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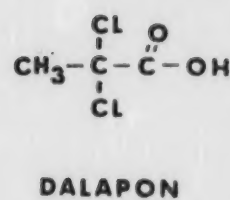
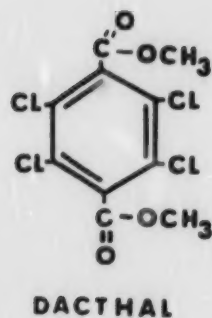
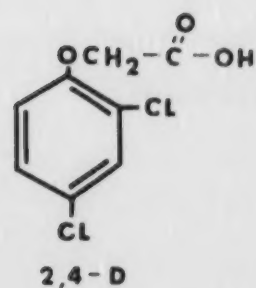
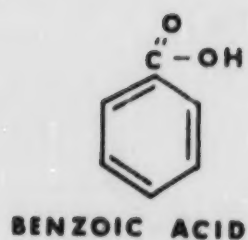
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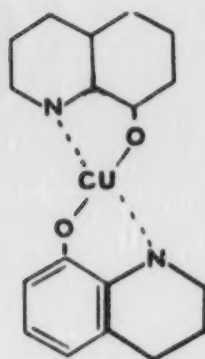


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STAM

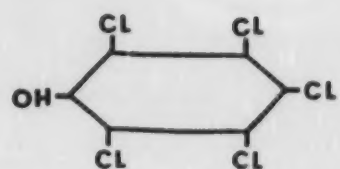




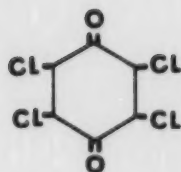
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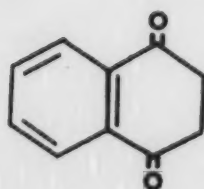
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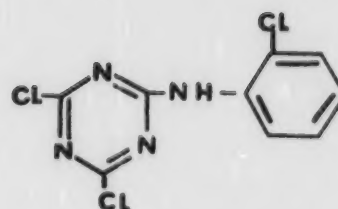
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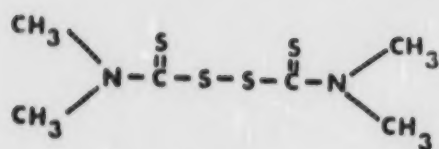
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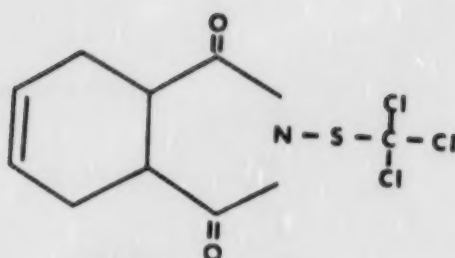
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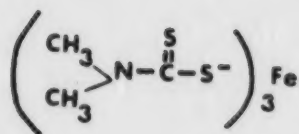
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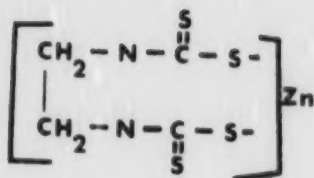
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CAPTAN

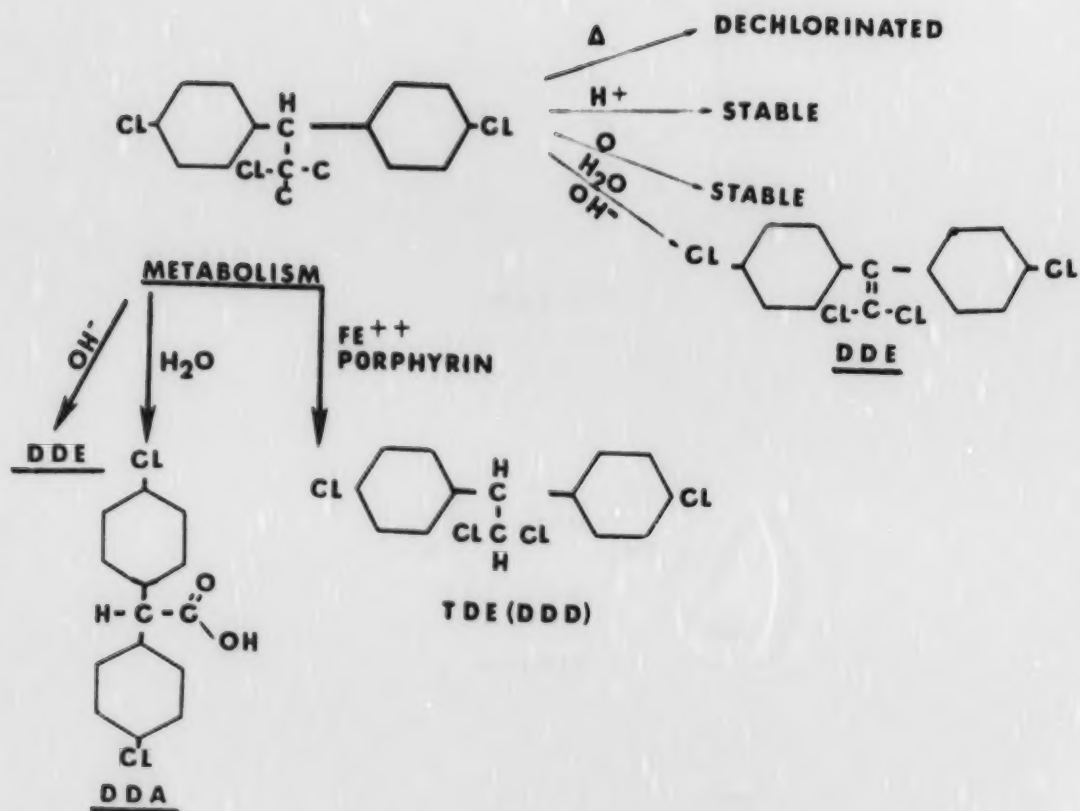


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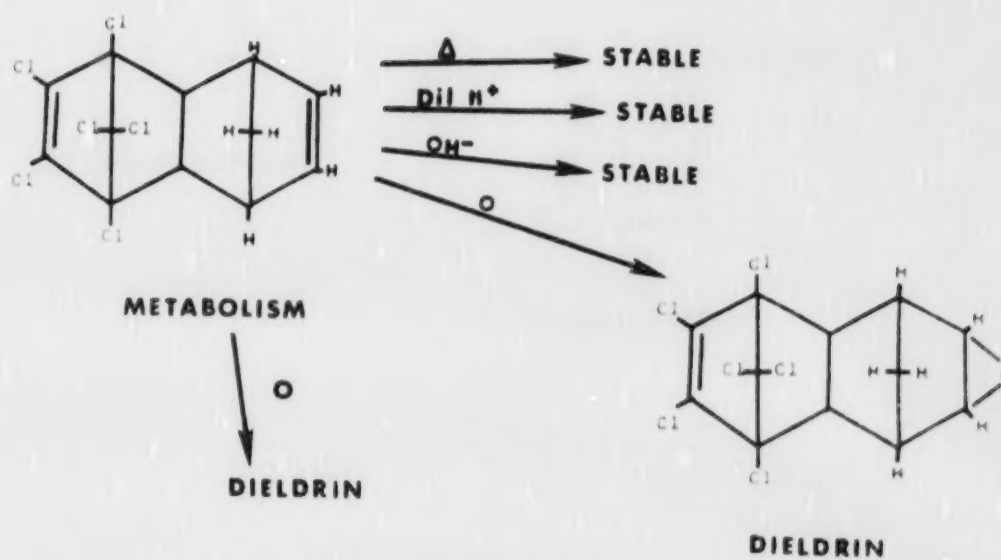


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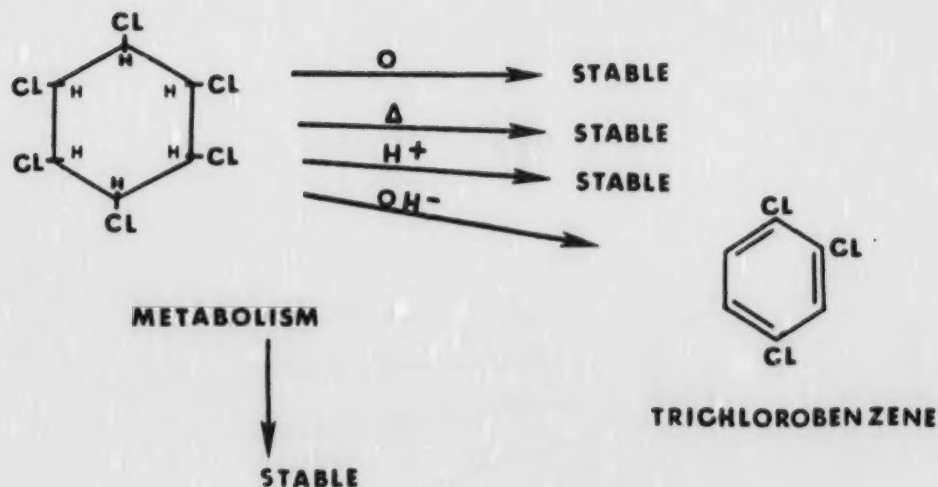
REACTION OF DDT



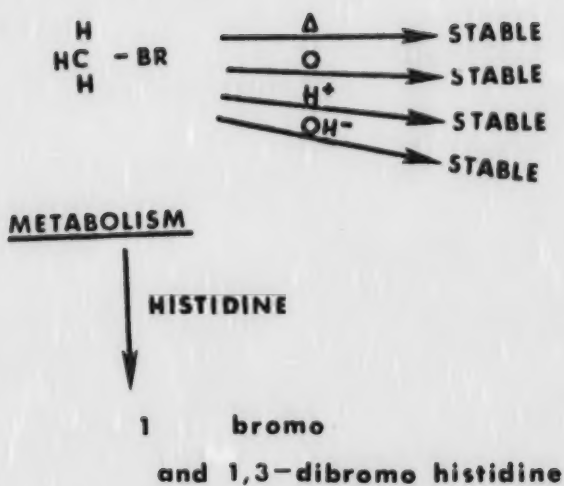
REACTIONS OF ALDRIN



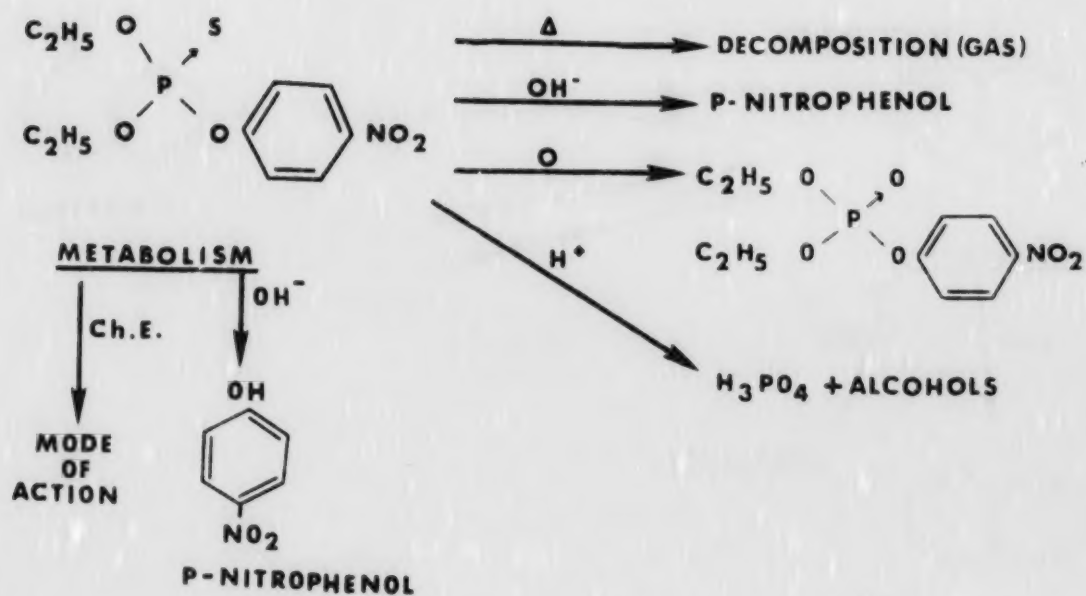
REACTIONS OF LINDANE



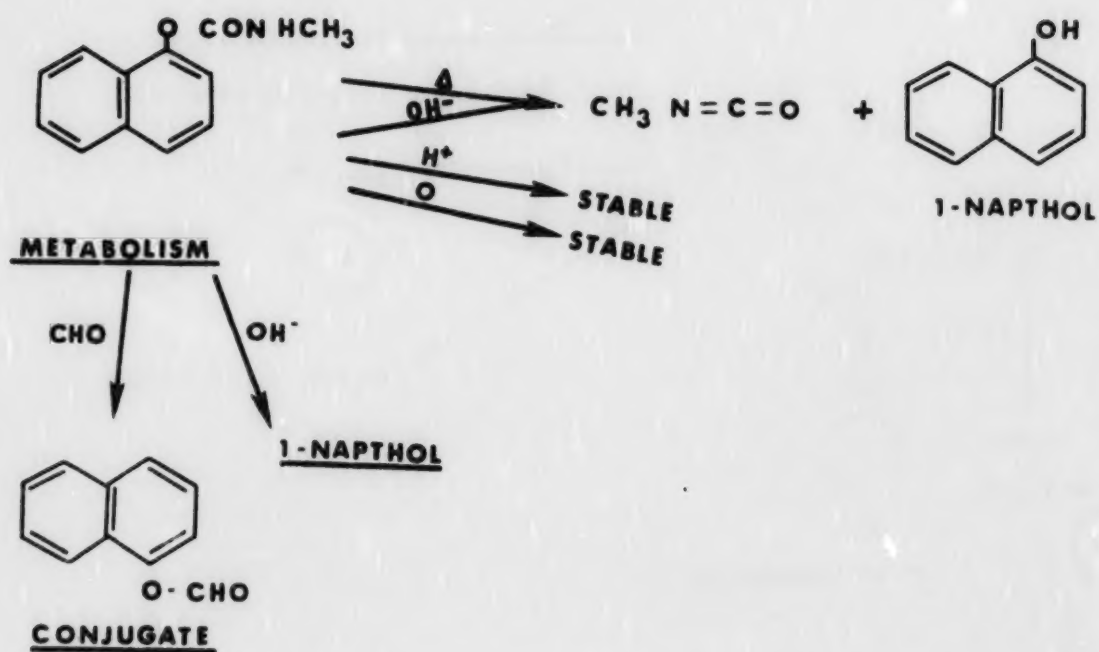
REACTIONS OF METHYL BROMIDE



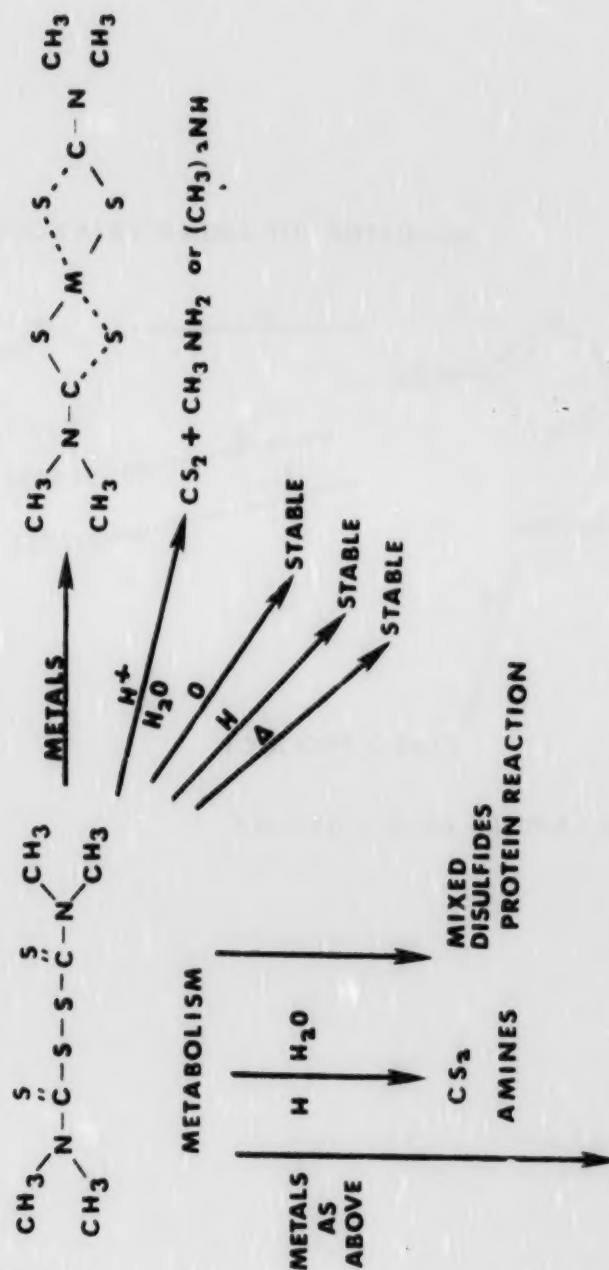
REACTIONS OF PARATHION



REACTIONS OF CARBARYL (SEVIN)

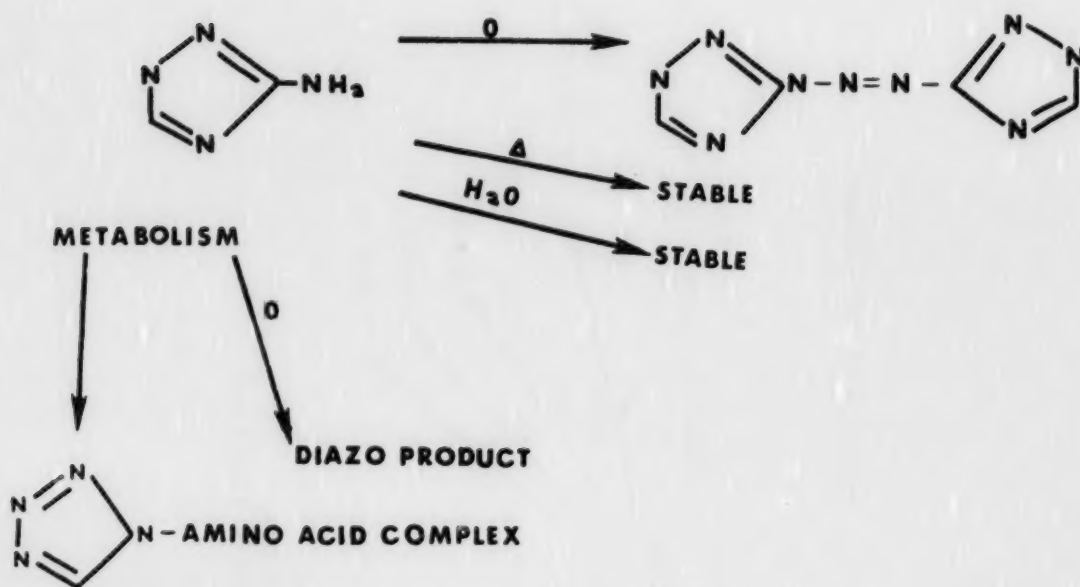


REACTIONS OF THIRAM

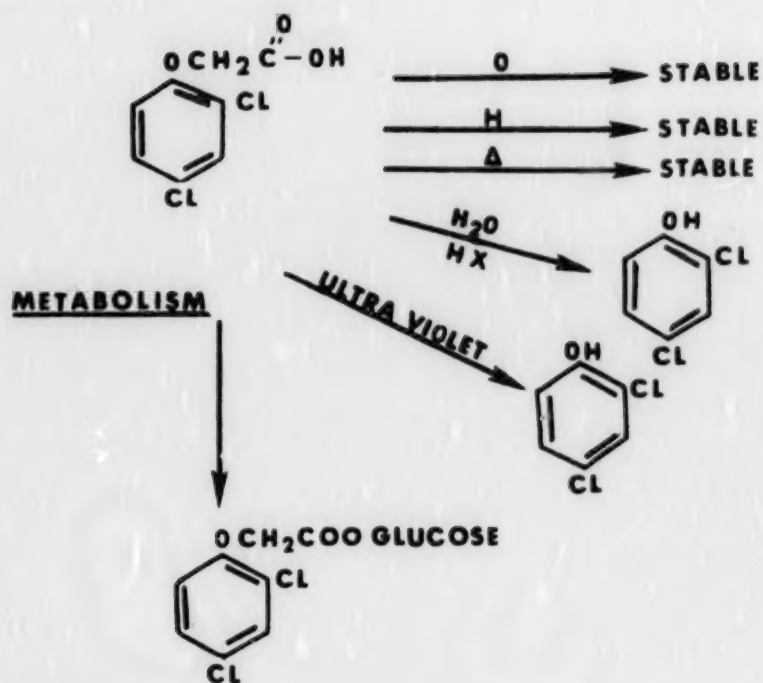


DHEW, PHS, CPEHS, FDA

REACTION OF AMINO TRIAZOLE



REACTION OF 2,4-D



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This outline was prepared by L.A.
Richardson, Training Supervisor, PRL,
Perrine, Florida.

BEHAVIOR OF PESTICIDES IN THE AQUATIC ENVIRONMENT

- I Many different materials have pesticidal effects although the chemicals vary in characteristics.
 - A Primary interest in water pollution control lies among chemicals that show:
 - 1 High toxicity at low concentrations
 - 2 Marked resistance to biological, chemical or physical alteration.
 - 3 Properties enhancing movement and contact with organisms.
 - B Chlorinated hydrocarbons exhibit all of the items in I. A to a degree that is disturbing.
 - C Other pesticides may have comparable toxicities but most lack the marked residual effect because they hydrolyze, oxidize or otherwise lose pesticidal effectiveness more rapidly in aquatic systems.
- II Pesticide formulations generally include a complex mixture; each component contributes some effect upon subsequent behavior in water.
 - A Usually each formulation includes more than one pesticide.
 - 1 Some insecticides immobilize quickly but have a high incidence of recovery.
 - 2 Other insecticides kill slowly.
 - 3 Pesticides generally show some selectivity such as that among insecticides and herbicides in general and for particular types within the general grouping.
 - B High toxicity generally means the use of a liquid or solid diluent in the formulation to avert necessity for highly precise and small measurements in dosage control.
 - C Formulations generally contain items promoting applicability for use.
 - 1 Wetting, spreading or emulsifying agents are commonly used in formulations.
 - 2 Components promoting shelf storage are added to minimize caking, separation, hydrolysis, etc.
 - 3 Oils, soaps or other materials may be used to promote adherence to surfaces after application.
 - 4 Components may be added such as for pH control of the formulation, or applied pesticide layer, or otherwise improve acceptability of the product in application, pesticidal effectiveness, or sales appeal.
- III Solubility of the pesticidal agent has a major effect upon distribution dynamics.
 - A Solubility is difficult to determine for a sparsely soluble material because of the effects of solubilizing agents, possible colloidal dispersion, and other conditions involved. Considering the chlorinated hydrocarbon group as an example:
 - 1 Lindane solubility in water appears to be about 3 mg/l.
 - 2 Heptachlor and gamma chlordane solubilities in water are about 10 micrograms/liter.

- 3 Dieldrin solubility in water is about 150 micrograms/liter.
- B Wilroy (Proc. 18th Industrial Waste Conference, Purdue University, Eng. Ext. Series 115, p. 413, 1964) reported a rug treating plant treatment operation with 12 to 65 mg of dieldrin per liter of lagoon influent and about 0.25 mg/l in the effluent.
- C Unpublished laboratory results in river water dosed with various chlorinated insecticides at 1 mg/l indicated that:
- 1 99% of the recovered lindane appeared in the liquid phase
 - 2 95 to 99% of chlordane and heptachlor recovered appeared in the silty fraction.
- D These data are in line with solubilities.
- IV Okey and Bogan (JWPCF, 37: No. 5, 692, May, 1965) reviewed apparent persistence of certain pesticides and relations in microbial metabolism.
- A Dieaway has been measurable on a long term basis (Pesticides in Soil and Water, an annotated bibliography, July 1964, Env. Health Series, WS&PC, Department of Health, Education and Welfare, Public Health Service).
- B It has been established that soil dieaway involves certain mechanisms that promote more rapid disappearance than that in water. These mechanisms are not clearly understood.
- 1 Ludzack and Mandia reported (Proc. 16th Industrial Waste Conference, Purdue University, Eng. Ext. Series 109, p. 540, 1962) results from 3 amino triazol where 3 AT dieaway in soils was at least 50 times the rate of dieaway in water.
- 2 Results on chlorinated insecticides tested (unpublished) definitely indicated dieaway but microbial degradation was not established.
- V Preference shown by pesticides for favoring concentration on hydrosols, organic material, and other surfaces in the aquatic environment has a large effect upon distribution. The general term sorption is preferable here since the effect may involve concentration on interfaces or within the mass by chemical or physical interrelations.
- A III. B and C indicate the degree of effect for river silt and detritus associated with an industrial wastewater.
- 1 Unpublished data on dieldrin filtrates (laboratory tests in river water) showed that added nutrients increased cell mass rapidly and decreased the dieldrin content of filtrates. The dieldrin content of filtrates increased in the same system with cell mass dieaway.
 - 2 The Lower Mississippi River Project (rep. in prep) observed 0.4 to 2.4% endrin and dieldrin in certain interceptor sewer deposits. Feedback to the following wastewater contributed to River Water concentrations above 4 micrograms of endrin per liter before correcting for sewer deposits and 0.3 micrograms of endrin/l in the river after correcting for feedback.
- B Surface active agents are added in formulations and commonly occur in surface water from natural or man-made activities. These can serve as concentrating agents on solid-liquid or liquid-gas interfaces.
- C The presence of oily materials of natural or man-made origin favor concentration because pesticide solubilities are many-fold greater in organics than in water. The net effect is to enhance distribution at the interface zone among solids, liquids and gases.

- VI Volatilization of pesticides from clean surfaces is relatively large, frequently amounting to 50 to 90% per day at summer temperatures.
 - A Contaminated surfaces imparting one or more sorption effects alter volatility effects.
 - B Concentrations of chlorinated insecticides in water (below solubility limits) were reduced as much as 70% by moderate rate aeration.
 - C Releasing air flow over the top of an unbroken surface resulted in greater insecticide losses than the same air flow released below the aqueous surface.
- VII Early efforts in pesticide studies were confused by the inability to obtain a material balance among dosage and recovery.
 - A Accountability has improved and with it an improved picture of behavior. Some of the factors affecting behavior include:
 - 1 The fraction of the system pesticide occurring as a surface slick, bed load or boundary deposit, or in the moving water.
 - 2 The interchange possibilities as related to solubility, associated contaminants and mixing and contact variables.
 - 3 The chemical or biological conversion possibilities.
 - 4 The possible complexation or sorption effects tending to immobilize or limit toxic action.
 - 5 Volatility interrelations.
 - B Answers on behavior are not easy to obtain and generally are clouded by uncertainties. Progress is possible as long as the overall pattern is checked instead of one or two phases of it.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, OWP, Cincinnati, Ohio 45226.

USE OF EXTRACTION METHODS IN PESTICIDE ANALYSIS

Extraction methods are used both as a means of concentration and as a means of purification. In general the less polar the compound, the easier it is to concentrate and purify. The chlorinated insecticides are quite non-polar and very easy to process. Phosphates also are satisfactory, although less so than the chlorinateds, and there is a wider range in properties for the members of this class. Carbamates and other insecticides are likely to be still more polar and difficult to process.

I EXTRACTION OF INSECTICIDES FROM SOLIDS

A Animal Tissues

A variety of treatments are in use, differing with the type of tissue. Body tissues may be extracted in the frozen condition or at the boiling point of the solvent or solvent mixture. Drying agents may be added. The major problem is to secure penetration of the wet tissues with organic solvents. It is very difficult to devise a method for determining the efficiency of this step.

B Mud and Soil

These are usually dried and extracted in some form of continuous extractor, such as the Soxhlet. The operation is easily carried out as compared with animal tissues.

C Fruits and Vegetables

A great deal of attention has been given to this problem by the Food and Drug Administration and many publications are available. Good recoveries can often be obtained by proper choice of solvent and conditions but clean-up may be difficult.

II EXTRACTION OF INSECTICIDES FROM WATER

A Planning the Sampling Step

An essential preliminary is to decide on the lower limit of interest as far as the insecticide is concerned. When this information is collated with the sensitivity of the analytical method, the required volume of sample can be calculated.

B Extraction from Water

1 Choice of solvent system

The solvent system must be chosen on the basis of the partition coefficient. A two-phase system is required, therefore the organic phase may be anything from butanol to hexane. The less polar the organic phase, the fewer interferences are likely to be extracted so hexane or other aliphatic hydrocarbons are usually best if the partition ratio is high enough. Chlorinated insecticides can usually be extracted very nicely with hexane. Phosphates are more polar and CHCl_3 may be necessary with some. For carbamates, etc., it will be necessary to consult the literature or try bench tests with common solvents, e.g.: CHCl_3 , ethyl acetate, methyl ethyl ketone, butanol, etc. Often NaCl or acids or bases can be added to change the partition ratios.

2 Determination of partition coefficient (PC)

A useful approximation to the PC can be obtained by equilibrating a realistic amount of insecticide with solvent and water in a separatory funnel. The PC is equal to the ratio of the concentrations of insecticide in the two phases.

3 Choosing the extraction equipment

The volume of water required and the PC determine the choice of extraction equipment.

a A separatory funnel is adequate for small volumes of water if not more than 1 or 2 serial extractions are required. The upper limit is probably on the order of 3 or 4 liters, although this can be increased at the expense of efficiency.

b Where only a small volume of water is sufficient but where the PC is unfavorable, small bench-type continuous extractors may be used. Because the solvent is recirculated the volume is low but the efficiency is high.

Such extractors may also be used with slurries of mud in water, etc.

c Large continuous extractors

These are bulky, expensive, and fragile but do permit the extraction of carboy size samples. (An interesting sidelight is to think of fish and crustacea as continuous extractors).

4 Examples of planning an analysis (Table 1)

III CLEAN-UP BY EXTRACTION METHODS

A Solubility of Solids

A concentrate from a large extractor usually contains much very polar, highly colored material. Most of this can be eliminated by digestion with hexane, etc. It is often necessary to dissolve the gummy sample in a ml or two of MeOH before adding a large amount of hexane; this is done in order to get contact between the solids and the solvent.

B Partition

1 Separatory funnel operations

Partition of chlorinated insecticides between acetonitrile and hexane is a now classic method to separate them from animal fats and mineral oils. It will seldom remove every trace of oil but is the best method for separating grams of oil or fat from μg of insecticide.

2 Paper chromatography

This is considered to be largely a matter of partition, as opposed to adsorption or ion exchange. L.C. Mitchell of the Food and Drug Administration has done much to popularize the use of paper impregnated with solvents. Cellulose TLC employs the

Table 1.

	Dieldrin (by G.C.)	Endrin (by I.R.)	Schradan (by G.C.)
Concentration limit	1 ppb	0.1 ppb	1 ppb
Sensitivity of anal. method	1 $\mu\text{g}/\text{ml}$	10 μg	(est.) 10 $\mu\text{g}/\text{ml}$
Volume of H_2O	1 liter	100 liters	10 liters
Partition coeff.	$\sim 10^3$ (hexane)	$\sim 10^3$ (hexane)	~ 7 (CHCl_3)
Extraction	Sep. funnel 2 X 100 ml	Large cont. extractor	Large cont. extr. (Sep. funnel possible)

same principle. Paper chromatography is a good means of identification, besides being a means of purification.

3 Column partition chromatography

In this method an inert support is used to hold one phase stationary while the other flows past it. Silicic acid, celite,

and cellulose are commonly used supports. The stationary phase is usually the polar phase but may be the non-polar phase if the support is first made hydrophobic. CPC is more flexible than paper chromatography because of opportunities for varying the developing solvent, e.g., by gradient elution.

This outline was prepared by R. H. Burttschell, Former Chemist, Waste Identification and Analysis Activities, Research and Development, Cincinnati Water Research Laboratory, SEC.

USE OF EXTRACTION METHODS IN PESTICIDE ANALYSIS - FRUITS AND VEGETABLES

I SAMPLING

A Collection

- 1 Statistical for commercial lots
- 2 Cross-sectional for field samples

B Preparation for Analysis

- 1 Chlorinated and phosphate organic pesticides, carbaryl, and chlorophenoxy acids.
 - a Entire sample chopped
 - b Portion taken for analysis
- 2 Dithiocarbamate residue
 - a Sample wedged
 - b Portion taken for analysis

II EXTRACTION, ISOLATION, AND DETERMINATION

A Chlorinated and Phosphate Organic Pesticides

- 1 Extraction
 - a Acetonitrile-water
 - b Petroleum ether
- 2 Isolation by column chromatography
 - a 6 + 94 eluant (ethyl ether + petroleum ether)
 - b 15 + 85 eluant (ethyl ether + petroleum ether)
- 3 Detection by GLC

B Carbaryl (Sevin)

1 Extraction

- a Methylene chloride

2 Isolation

- a Coagulating solution (.5 g NH_4Cl in 400 ml. HOH containing 1 ml. H_3PO_4)
- b 10% Aqueous acetone
- c Methylene chloride

3 Determination

- a T. L. C.
- b Colorimetric - forms yellow dye when reacted with p-nitro-benzenediazonium fluoborate

C Chlorophenoxy Acids (Herbicides)

1 Extraction

- a 3 Organic solvent system (ethyl alcohol, petroleum ether, and ethyl ether)

2 Isolation

- a Alkaline extraction (4% NaHCO_3 solution)
- b Acidification with 10% H_2SO_4
- c Chloroform

3 Determination

- a Methylation with diazomethane
- b G. L. C.
- c T. L. C.

D Dithiocarbamate Residues

Extraction Methods in Pesticide Analysis

1 Isolation

- a Distillation with 10N H_2SO_4 decomposing residue and evolving CS_2
- b CS_2 filtered through 20% zinc acetate to remove interfering gases

2 Determination

- a Colorimetric - CS_2 reacts with color reagent (Cupric acetate monohydrate + diethanolamine) to form yellow cupric salt of N, N-bis (2-hydroxyethyl) dithiocarbamic acid

III PROBLEMS

A Chlorinated and Phosphate Organic Pesticides

1 Additional clean-up procedures

- a MgO-celite column

b Saponification

B Carbaryl (Sevin)

- 1 Care in filtration with hyflo-super cel

C Chlorophenoxy Acids (Herbicides)

1 Methylation

D Dithiocarbamate Residue

1 Distillation

2 Zinc acetate solution

This outline was prepared by Joseph E. Hayes, DHEW, Food and Drug Administration, Atlanta, Georgia.

THIN-LAYER CHROMATOGRAPHY

I INTRODUCTION

A Historical

- 1 Adsorption chromatography was discovered by Tswett in 1903. Tswett found that plant pigments (e.g., chlorophylls) can be separated by filtering a solution of them in petroleum ether through a column of calcium carbonate. He noticed that yellow and green zones were formed in the column.
- 2 Adsorption chromatography was introduced in 1931 when the method was used for the preparative chemistry of the polyene pigments, for example the separation of carotene into its components.
- 3 Partition chromatography for hydrophilic compounds was introduced in 1941.
- 4 Thin-layer chromatography was introduced by Stahl in 1956. He described an ingenious and practicable device for preparing layers of silica gel about 250 microns thick with a plaster of paris binder.
- 5 Thin-layer chromatography has proven useful for the quantitative estimation of pesticides and for the "clean-up" of sample aliquots prior to the estimation of their components by gas chromatography.

B Theory

- 1 Thin-layer chromatography is an analytical technique which has proven useful for the separation of components in a given system and to evaluate the components in that system.
- 2 All of the techniques of chromatography are based upon the same simple principle. They involve a moving system of some type (liquid or gas) which is in

equilibrium with a stationary phase. When the stationary phase is a solid and the forces acting between it and the mixture are adsorptive in nature, the technique is called adsorption chromatography.

- 3 When the stationary phase is a simple liquid or a liquid held on some type of support, the chromatography is considered to be partition chromatography.
- 4 Diagnostic or qualitative thin-layer chromatography is used to determine the number of components in a system.
- 5 Preparative thin-layer chromatography is used to separate a mixture into its components in such a way that reasonable amounts can be isolated and studied.
- 6 Quantitative thin-layer chromatography is used to measure the amount of the components present. This is done by estimating the density of the spots as compared to standard spots.

II DEFINITION OF TERMS

- A Adsorbent. The finely divided powder which makes up the stationary phase in adsorption chromatography, or holds the stationary liquid in partition chromatography.
- B Layer. The thin layer of adsorbent bound or unbound, deposited on a glass plate.
- C Spotting. The application of the substance to the thin layer adsorbent.
- D Development. The passing of a liquid through the layer to effect a separation.
- E Developer. The liquid used for the development.
- F Eluent. The complete removal of a substance from the adsorbent layer.

Thin-Layer Chromatography

G Visualization. The rendering visible of the results of a developed chromatogram.

H Ratio factor " R_f ". The distance traveled by a given substance divided by the distance traveled by the solvent front. Both measured as the origin.

III ADSORBENTS

A Silicic Acid or Silica Gel

Chemically identical, but the gel is prepared in such a manner as to enhance its adsorptive power.

B Aluminum Oxide or Alumina

C Diatomaceous Earth

D Powdered Cellulose

E Essentially any substance which has desirable adsorptive and chemical characteristics can be successfully used in thin-layer chromatography.

IV PREPARATION OF PLATES

A Methods

- 1 Spreading or spraying of slurries
- 2 Dipping in a slurry

B Equipment

- 1 Kirchner type
- 2 Stahl type
- 3 Microchromatoplates

C Layer thickness

- 1 For qualitative analysis thin layers (250 microns) are used.
- 2 For preparative thin layer thicker layers may be used.

D Moisture Content

- 1 Trace quantities of water greatly reduce the activity of various adsorbents.
- 2 Normally plates are activated by drying at 110°C for one hour and then stored in a desiccator.

V TECHNIQUE

A Spotting the Plate

- 1 The activity of the layer is affected by humidity and temperature.
- 2 The sample is applied so that the direction of the development is opposite to the direction in which the layer has been applied.
- 3 Use of spotting template
- 4 Use of micro-pipette and micro-liter syringe
- 5 Size of sample spots
- 6 Use of standards

B Solvent System

- 1 Investigation of single and multi-component systems
- 2 Carbon tetrachloride, hexane, and n-heptane as solvents for separation of pesticides

C Development of the Plate

- 1 Development tank
- 2 The plate is placed in a glass tank containing the solvent. When the solvent front reaches the upper reference line (10 cm) the plate is removed and the solvent is allowed to evaporate.

VI VISUALIZATION

A Rhodamine B

- 1 Spray reagents - Rhodamine B, spirit soluble, 0.1 mg per ml in ethanol
- 2 Method of spraying
- 3 Advantages of Rhodamine B
 - a The pesticide is not destroyed.
 - b The exact position of the pesticide can be determined.
 - c The use of a selective solvent permits the pesticide to be eluted from the adsorbent while the Rhodamine B is retained.

B Impregnation of Plates with AgNO_3

- 1 Increased sensitivity for quantitative estimation
- 2 Elimination of spraying

C Exposure to UV Light

- 1 Pesticides appear as quenched areas in the fluorescent background.

- 2 Recovery studies using Rhodamine B spray are comparable to those using silver nitrate spray. The following pesticides may be seen at the 10 μg level under UV light after Rhodamine B spray:

endrin	heptachlor
dieldrin	heptachlor epoxide
DDT	methoxychlor
aldrin	toxaphene
lindane	DDE
chlordane	ovex
parathion	tedion
methyl parathion	

- D Suggested systems for adsorption chromatography are given in Table 1.

E Recovery

- 1 Elution of pesticides from the thin layer with a mixture of ethyl and petroleum ethers.
- 2 Recovery zones as shown in Figure 1.
- 3 Silica gel collection assembly - Figure 2.

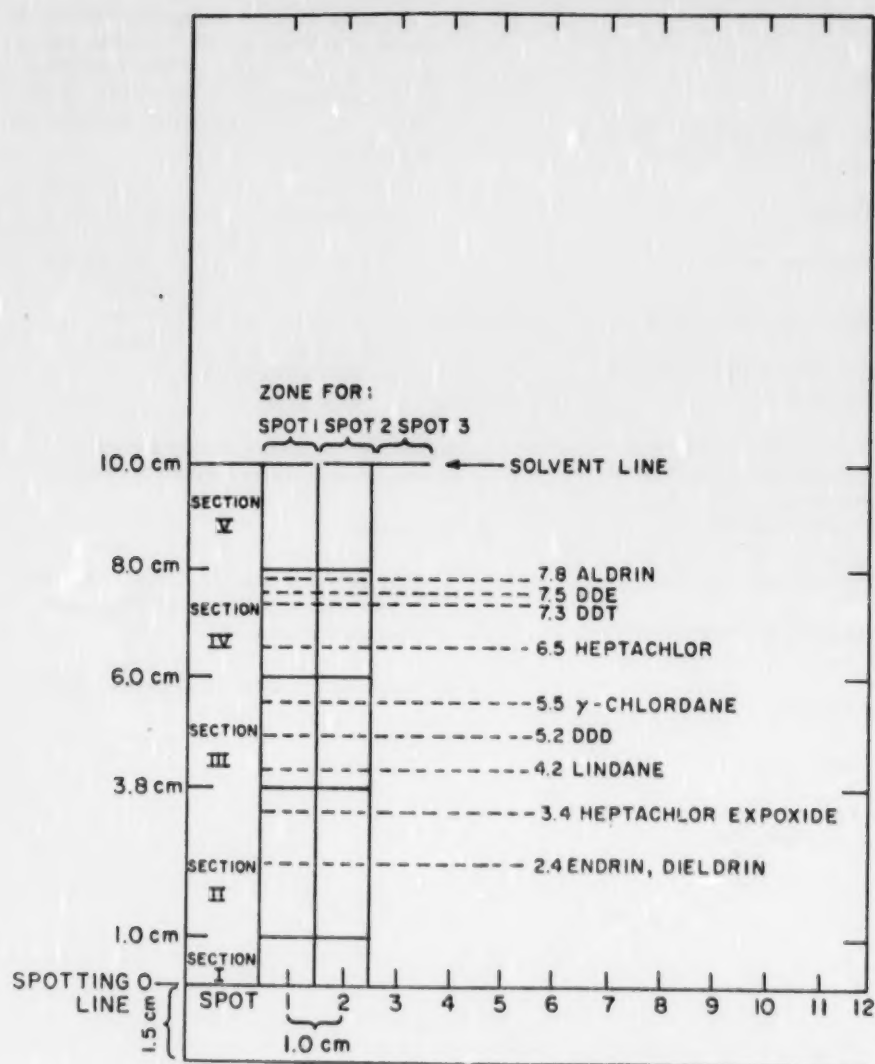
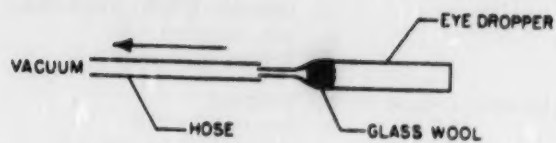


Figure 1. Diagram of Designation of Sections in the Cleanup and Separation of CCE-Aromatics on Silica Gel Layers.



SILICA GEL COLLECTION ASSEMBLY

FIGURE 2

TABLE 1
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
1. Long-chain (over eight carbons) aliphatic ketones	silica gel G	a. benzene-EtOEt mixtures b. toluene-EtOEt mixtures c. pet. ether-EtOEt mixtures	phosphomolybdic acid (2, 4-DNPH)
2. Aliphatic and aromatic aldehydes and ketones	aluminum oxide	a. benzene b. benzene-EtOH (98:2) (95:5) (90:10) c. CHCl_3 d. EtOEt e. pet. ether-benzene (1:1)	2, 4-DNPH
3. Aromatic aldehydes and ketones	silica gel G	hexane-EtOAc (4:1) (3:2)	(U. V. on phosphors)
4. Vanillin and other substituted benzaldehydes	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2) c. CHCl_3 -EtOAc (98:2) d. decalin- CH_2Cl_2 -MeOH (5:4:1)	(U. V. on phosphors)
5. 2, 4-Dinitrophenylhydrazones of aldehydes	silica gel G	a. benzene-pet. ether (3:1) for aliphatic b. benzene-EtOAc (95:5) for aromatic	colored
6. Miscellaneous alkaloids	silica gel G	a. CHCl_3 -acetone-diethylamine (5:4:1) b. CHCl_3 -diethylamine (9:1) c. cyclohexane- CHCl_3 -diethylamine (5:4:1) d. cyclohexane-diethylamine (9:1) e. benzene-EtOAc-diethylamine (7:2:1)	(Dragendorff's Reagent)
	aluminum oxide	f. CHCl_3 g. cyclohexane- CHCl_3 (3:7) plus 0.05% diethylamine	
7. Alkaloids and barbiturates in toxicology	silica gel G	a. MeOH b. CHCl_3 -EtOEt (85:15)	(Dragendorff's Reagent)
8. Strong-base amines	unbound aluminum oxide	a. acetone-heptane (1:1) b. $\text{CHCl}_3/\text{NH}_3$ (sat. at 22°)-EtOH (96%) (30:1)	I_2 vapor-U. V. (Dragendorff's Reagent)
9. Sugar acetates and inositol acetates	silica gel G	benzene with 2-10% MeOH	H_2O

TABLE 1 (continued)
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
10. Aldose 2, 4-DNPH's	a. aluminum oxide G b. silica gel G	a. toluene-EtOAc (1:1) b. toluene-EtOAc (3:1) (1:1)	a. colored compounds b. NaOH
11. Cardiac glycosides	silica gel G	a. CHCl_3 -MeOH (9:1) b. CHCl_3 -acetone (65:35)	H_2SO_4 and heat
12. Dicarboxylic acids	silica gel G	a. benzene-MeOH-HOAc (45:8:4) b. benzene-dioxane-HOAc (90:25:4)	bromphenol blue acidified with citric acid
13. p-Hydroxybenzoic acid esters	silica gel G	pentane-HOAc (88:12)	(U.V. on phosphors)
14. Sulfonamides	silica gel G	CHCl_3 -EtOH- heptane (1:1:1)	p-dimethylamino-benzaldehyde, acidified (U.V. on phosphors)
15. Food dyes	silica gel G	a. CHCl_3 -acetic anhydride (75:2) b. benzene c. methyl ethyl ketone-HOAc-MeOH (40:5:5)	SbCl_3 in CHCl_3
16. Misc. essential oils	silica gel G	benzene- CHCl_3 (1:1)	SbCl_3 in CHCl_3
17. Coumarins	silica gel G	a. pet. ether-EtOAc (2:1) b. hexane-EtOAc (5:2)	(U.V. on phosphors)
18. Alkali metals- Na^{1+} , Li^{1+} , K^{1+} , Mg^{2+}	purified silica gel G	EtOH-HOAc (100:1)	acid violet (1.5% aqueous soln.)
19. 24 ferrocene	silica gel G	a. benzene b. benzene-EtOH (30:1) (15:1) c. propylene glycol-MeOH (1:1) d. propylene glycol-chlorobenzene-MeOH (1:1:1)	(U.V. on phosphors)
20. Misc. lipids	silica gel G	a. EtOEt b. isopropyl ether c. isopropyl ether-HOAc (98.5:1.5)	(H_2SO_4 and heat)
21. Fatty acid methyl esters	silica gel G	hexane-EtOEt mixtures (up to 30% EtOEt)	a. I_2 b. H_2SO_4 and heat
22. Glycerides	silica gel G	a. Skellysolve F-EtOEt (70:30)	H_2SO_4 and heat

TABLE 1 (continued)
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
		b. (10:90) for monoglycerides c. (70:30) for diglycerides d. (90:10) for triglycerides e. (60:40) (35:65) (85:15)	
23. Long-chain aliphatic alcohols	silica gel G	a. pet. ether-EtOEt-HOAc (90:10:1) b. pet. ether-EtOEt (20:80) (10:90) (70:30)	H ₂ SO ₄ and heat
24. Phenols	a. starch-bound silicic acid kieselguhr (1:1)	a. xylene b. xylene-CHCl ₃ (3:1) (1:1) (1:3) c. CHCl ₃	(U.V. on phosphors)
25. Phenols and phenolic acids	starch-bound silicic acid with phosphors	a. Skellysolve B- EtOEt (3:7) b. Skellysolve B- EtOAc (1:3) c. Skellysolve B-acetone (3:1)	(U.V. on phosphors)
26. Phenols	plaster-of-Paris bound silica gel	a. hexane-EtOAc (4:1) (3:2) b. benzene-EtOEt (4:1) c. benzene	(U.V. on phosphors)
27. 3,5-Dinitrobenzoates of alcohols and phenols	silica gel G	a. benzene-pet. ether (1:1) b. hexane-EtOAc (85:15) (75:25) c. toluene-EtOAc (90:10)	colored compounds
28. Steroids	silica gel G	a. benzene b. benzene-EtOAc (9:1) (2:1) c. cyclohexane-EtOAc (9:1) (19:1) d. 1,2-dichloroethane	SbCl ₃ in CHCl ₃
29. 19-Nor-steroids	silica gel G	EtOAc-cyclohexane mixtures	SbCl ₃ in CHCl ₃
30. Blood cholesterol and cholesterol esters	silica gel G	a. benzene b. benzene-EtOAc (9:1) c. 1,2-dichloroethane d. CHCl ₃	SbCl ₃ in CHCl ₃
31. Triterpenoids	silica gel G	a. isopropyl ether-acetone (5:2) (19:1) b. isopropyl ether c. cyclohexane d. benzene e. CH ₂ Cl ₂	(H ₂ SO ₄ and heat)

TABLE 1 (continued)
SUGGESTED SYSTEMS FOR ADSORPTION CHROMATOGRAPHY

Compounds	Adsorbent	Developer	Visualization
32. Carotenes and fat-soluble vitamins, A, D, E, K	unbound aluminum oxide	a. MeOH b. CCl_4 c. xylene	H_2SO_4 and heat
33. Thiophene derivatives	a. silica gel G b. aluminum oxide G	a. benzene- CHCl_3 (9:1) b. MeOH c. pet. ether	(U.V. on phosphors)
34. Plasticizers (phthalates, phosphates and other esters)	silica gel G with phosphors	a. isooctane-EtOAc (90:10) b. benzene-EtOAc (95:5) c. butyl ether - hexane (80:20)	a. (U.V. on phosphors) b. I_2

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GAS CHROMATOGRAPHY

Historically, the science of chromatography can be traced or attributed to the initial paper chromatographic separation process reported by Runge in 1850, and the Polish biochemist Iswett is considered to be the founder of column chromatography.

There are four major chromatographic processes as we know them today: 1) gas-liquid chromatography (GLC), 2) gas-solid chromatography (GSC), 3) liquid-liquid chromatography (LLC), and solid-liquid chromatography (SLC).

The name is somewhat misleading; there is nothing chromatic about the method or its results. The name comes from the original method of liquid-solid chromatography described by Iswett.

In gas chromatography the compounds in a mixture migrate at differing speeds when carried along by an inert gas through a tube that has been packed or treated in a special way. This method was developed by A. T. James and A. J. P. Martin. Gas chromatography is a general term which covers both gas-liquid and gas-solid chromatography.

The basic requirement for a sample to be chromatographed is that the sample must be volatile, or if enough heat is added, it will volatilize. The sample must not decompose in the sampling step; it must remain stable.

Since the vaporization of the sample occurs in an inert atmosphere, decomposition of the sample is generally not a significant problem. A solid can be chromatographed provided that a suitable volatile solvent can be found to dissolve it and that the solution can be vaporized.

GLC can be readily compared to an analytical distillation; however, it is a much more efficient method of separation. Where a good distillation column may operate with 100-200 theoretical plates, a mediocre GLC column may operate with 1000 theoretical plates,

and 500,000 plates have been obtained. It should be pointed out that one distillation plate is equal to 10 GLC plates. Where a micro-distillation column would use a minimum charge of 10 mls, a GLC column would use 0.001 ml. Where a distillation unit would separate compounds by differences in boiling points and be troubled by azeotropes, a chromatographic column can separate according to boiling points or by other molecular properties and it is not bothered by azeotropes. Very simply, the chromatographic system consists of 1) a supply of compressed gas (usually nitrogen or helium) called the carrier gas, which is the mobile phase; 2) a chromatographic column; and 3) a detector. The detector and column are normally housed in a thermostated oven to regulate their temperature. The output of the detector is fed to a millivolt recorder.

The chromatographic column, the heart of the system, consists of a metal or glass tube filled with a packing. This packing consists of an inert support coated with a high-boiling organic liquid. This liquid is known as the liquid phase, and it is the stationary phase. It is this liquid phase that causes separation in mixtures. The liquid must have a low vapor pressure so that it is not swept out of the column by the carrier gas.

The detector is located at the exit of the column; it allows us to see what separations have been accomplished by the column. There are now in existence many types of detectors. These include the thermal conductivity, flame ionization, electron capture, thermionic, flame photometric, microcoulometric, and electrolytic conductivity detectors.

For a given column operated at a given temperature and flow, the length of time that each component spends in the column, called the "retention time", is a constant. If the sample is injected a second time into

the column, the chromatograms will be identical, since the retention times are constant.

Let us look more closely at what happens to a collection of molecules injected into the carrier gas moving through the column of a gas chromatograph. Some of the molecules rapidly dissolve in the liquid phase, equilibrium is soon established as they pass back and forth between the liquid and vapor filling the interspaces of the column packing. At equilibrium the concentration of molecules of each type is a constant ratio in the two phases.

Molecules of compound A, for example, may partition themselves equally between the liquid and vapor phase; molecules of compound B, on the other hand, may be highly soluble in the liquid phase and therefore relatively few of them will be found in the vapor phase once equilibrium is reached. In this case the carrier gas will tend to sweep molecules of compound A down the tube, leaving those of compound B behind in the liquid. Once the molecules of A have been carried to a section containing fresh liquid, however, some of them will redissolve until a new equilibrium is reached. Likewise, when fresh gas passes over the liquid containing molecules of B in solution, some of the B molecules will enter the gas phase in order to establish equilibrium. If we regard the sample as a plug of molecules moving through the column, we can visualize a sort of molecular leapfrog. The volatile molecules are continuously being swept to the head of the plug, where they redissolve; the less volatile molecules fall to the tail of the plug, but are continuously being picked up and inched forward by the gas stream pressing from behind. Finally, if conditions are right, all the molecules of the more volatile components will be carried clear ahead of the less volatile and a clean separation will be achieved. NOTE DIAGRAM.

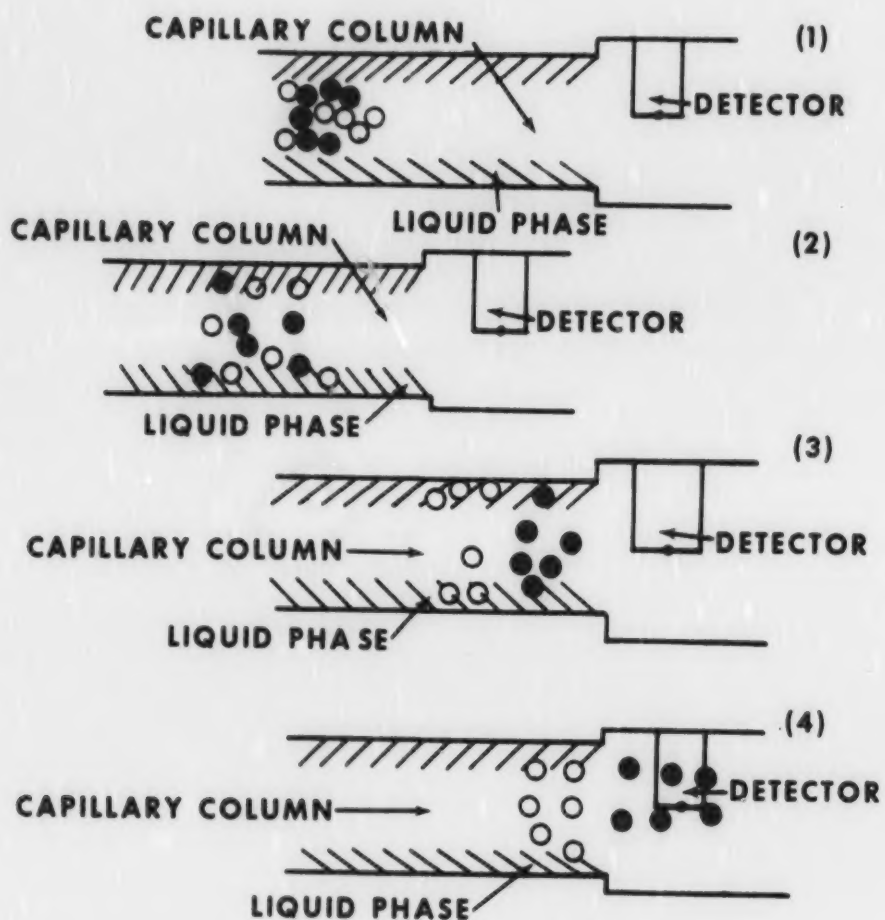
Another approach is to make up synthetic mixtures of the sample under investigation. The mixtures are run under the same set of conditions. For each component one obtains a calibration curve by plotting the peak height versus the concentration of the component in the sample. To analyze a mixture of unknown concentrations, the sample is chromatographed and the peak height for each component is found. Using the calibration curves, one can determine the concentration of each component. This method is dependent upon sample size since the peak height is a function of both the concentration of a component and the size of the sample.

By altering the nature of the liquid phase, it is possible to alter its interaction with the components being separated. By this method, changes in the relative volatility are affected, making difficult separations very easy.

The selectivity of the liquid phase and the extremely high efficiency of the chromatographic column allow one to separate a large number of mixtures.

When dealing with wide boiling mixtures, a problem arises as to the best temperature to operate the column. If the column is operated at a low temperature, low boilers are well separated but the high boilers emerge as very broad peaks after a long period of time. If the column is operated at a high temperature, the retention time for the high boiler is reduced and the peaks are much sharper, but the low boilers emerge quickly with poor separation. This problem can be overcome by programming.

The proper selection and subsequent adjustment of the operating conditions for an analysis is one of the most important keys to success in gas chromatography.



In review we have looked at gas chromatography as a system for separating complex mixtures and by using it as a qualitative tool, to identify the components of the mixture.

This outline was prepared by William R. Davis, former Training Officer, Southeast Water Laboratory, College Station Road, Athens, GA.

INTRODUCTION TO GAS-LIQUID CHROMATOGRAPHY

Part I

I INTRODUCTION

A Definition

Gas-liquid chromatography is an analytical method for the separation and identification of a mixture of volatile (usually organic) components in a sample. As with any chromatographic technique the column consists of two phases, the immobile or stationary phase (a liquid on an inert solid support), and the mobile phase (an inert gas). The column functions to separate the sample components because they have varying vapor pressures and affinities for the stationary phase. In many ways the column behavior resembles that of fractional distillation. The partition which occurs between the mobile and immobile phases will thus cause the components to proceed through the column at varying rates. The separation is recorded and quantitated by the detector system.

B Advantages

- 1 GLC can be used to separate compounds of similar boiling points which cannot easily be separated by distillation. (See Table 1)
- 2 GLC can be extremely sensitive; for example, using the electron capture detector it is possible to "see" picogram (10^{-12}) quantities.

Table 1. SEPARATIONS BY GLC

Compounds	Reference
3-Methylcyclohexene (B.P. 104°C) and 4-Methylcyclohexane (B.P. 103°C)	Aerograph Research Notes (Spring 1964)
Cyclohexane (B.P. 80.8°C) and Benzene (B.P. 80.2°C)	Chromosorb Newsletter (FF-104)

C Disadvantages

- 1 Due to the extreme sensitivity possible it is often necessary to apply extensive cleanup techniques.
- 2 The many variables of the technique require a skilled analyst.

II COMPONENTS OF A GAS CHROMATOGRAPH (See Figure 1)

A Gas Supply

The mobile phase (carrier gas) transports the sample components through the column into the detector. The type of gas used varies with the detector. (See Table 2)

B Injector

Liquid samples are manually introduced into the heated injector block through a rubber septum by means of a syringe. Automatic liquid injectors as well as injection systems for solid and gaseous samples are commercially available.

C Column

The vaporized sample enters the column which can be glass or metal and of varying length (1' - 20') and diameter (1/8" - 1/4"). The column is packed with the stationary (immobile) phase and contained within a constant temperature oven.

1 Solid support

The solid support should have a large surface area yet be inert so that active sites will not cause adsorption of sample components. Diatomaceous earths, teflon and glass beads have been used. (See Table 3)

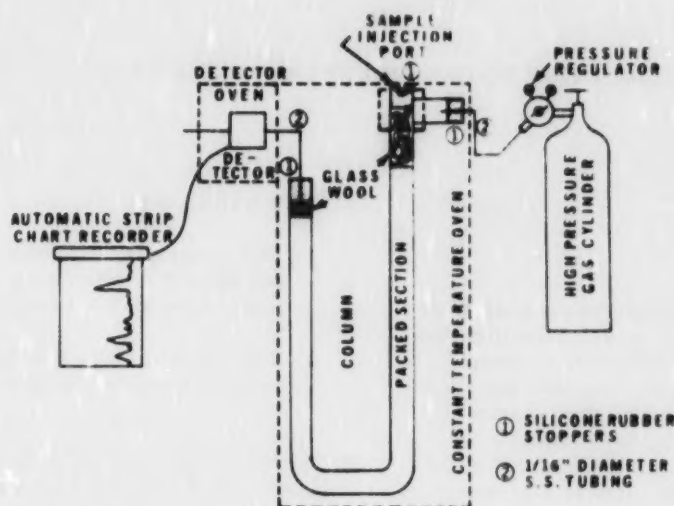
Figure 1. COMPONENTS OF A GAS CHROMATOGRAPH²⁰

Table 2. CARRIER GASES

Detector	Carrier gas
Thermal conductivity	Helium (Purified, Grade A)
Microcoulometric	Helium (Purified, Grade A)
Flame ionization	Hydrogen (Purified)
Electron capture	Nitrogen or a mixture of 95% argon and 5% methane (Purified)

Table 3. SOLID SUPPORTS

Support	Surface area (m ² /gm)
Chromosorb P (Diatomaceous Earth)	4.8
Chromosorb W (Diatomaceous Earth)	1.2
Chromosorb G (Diatomaceous Earth)	0.5
Chromosorb T (Teflon)	7.0 - 8.0

2 Stationary liquid

The separation and partition occurring in the column is directly affected by the choice of stationary liquid. For example, in the separation of benzene (B. P. 80.1°C) and cyclohexane (B. P. 80.8°C), the choice of a non-polar phase such as hexadecane results in benzene preceding cyclohexane off the column. However, if a more polar phase such as benzylbiphenyl is chosen cyclohexane precedes benzene. Table 4 shows some typical stationary liquids and their uses. (NOTE: One requirement for any liquid is that it have a high boiling point so that it will not boil off the column)

D Detector

The detector or brain of the gas chromatograph senses and measures the quantity of sample component coming off the column. The detector should be maintained at a temperature higher than the column so that condensation does not occur in the detector block. Several types of detectors are in use today.

*Reproduced (with permission) from Chemistry, (37:11, p. 13. November 1964).

Table 4. STATIONARY LIQUIDS

Stationary liquid	Used to separate
Silicone oils QF-1, Dow Corning 200, and Dow 11, OV-1, OV-3, OV-17, OV-Z10, OV-225	Chlorinated hydrocarbons pesticides
Silicone oil SE-30	Homologous series of n-alkanes
Benzyl-Cyanide-Silver Nitrate	Homologous series of olefins
Polyethylene Glycol	Amines
Cyano Silicone	Steroids

1 Thermal conductivity

This detector consists of a Wheatstone bridge two arms of which are thermal conductivity cells each containing a small heated element. When only carrier gas is flowing through both the sample cell and reference cell, the resistance of the heated element is constant in both cells. The bridge remains balanced and baseline is recorded. However, when carrier gas plus sample component enter the sample cell, the thermal conductivity in that cell changes thus also producing a change in the resistance of the heated element. The bridge becomes unbalanced and a peak is recorded. The main disadvantage of the TC cell in water pollution work is its lack of sensitivity.

2 Ionization detectors

a Flame

This detector consists of a flame situated between a cathode and anode. As carrier gas alone burns, some electrons and negative ions are produced which are collected at the anode and recorded as baseline. When carrier gas plus sample component are burned, more electrons and negative ions are produced which result in a peak on the recorder. The detector is capable of "seeing" nanogram quantities of organic compounds; however, the detector is sensitive to all organic compounds. This lack of specificity produces disadvantages in the analysis of water

extracts which contain a variety of naturally occurring organics.

b Electron capture (See Figure 2)

This detector consists of a radiation source (e.g., tritium) capable of producing slow electrons in a carrier gas such as nitrogen. The electrons collected at the anode are recorded as baseline. When sample components which have an electron affinity (e.g., chlorinated hydrocarbons) enter the detector, electrons are "captured." The subsequent decrease in current is recorded as a peak. The detector has the advantage that it is extremely sensitive (pico-gram range) and is somewhat selective.

c Thermionic

A recent adaptation of the flame ionization detector shows promise for the specific analysis of organic phosphorus compounds. An alkali salt is incorporated into the design of a conventional flame ionization detector so that the salt heated by the flame produces an ion current. When compounds containing phosphorus emerge from the column, they give 600X the response with this detector as with the conventional flame.

3 Microcoulometric

Although less sensitive (by approximately a factor of 10) than electron capture,

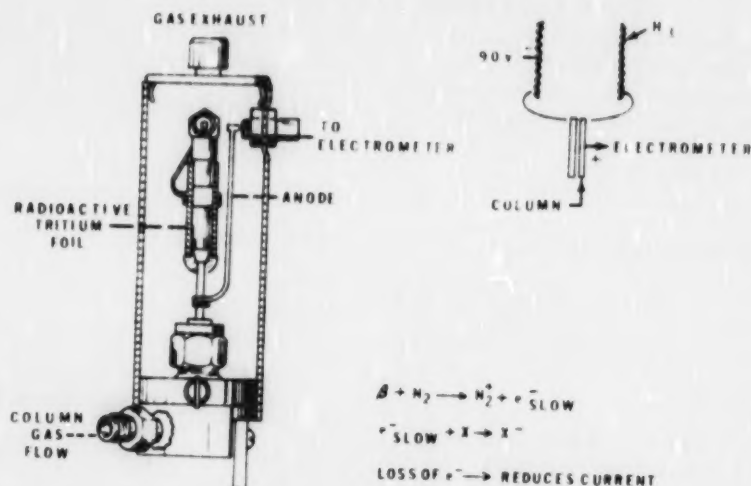


Figure 2. ELECTRON CAPTURE DETECTOR (Wilkens Instrument Company)

this detector is finding wide use in pesticide analysis. This highly specific detector consists of titration cells for the measurement of chloride-containing and sulfur-containing compounds. The sample component emerging from the column is combusted to produce HCl or SO₂, respectively. HCl is continuously titrated by silver ions present in the cell; the amount of current required to regenerate these silver ions is recorded as a peak. The system for sulfur containing compounds is analogous except that SO₂ produced is continuously titrated by I₂ which is subsequently regenerated.

Another microcoulometric detector has recently been applied to the specific determination of nitrogen. It is based on the reduction of nitrogen-containing compounds to NH₃ which is then titrated by H⁺ in the titration cell.

4 Coulson Electrolytic Conductivity

This detector was primarily developed for the detection of organic halides, organic nitrogen compounds, and organic sulfur compounds.

The unit consists of pyrolyzer with a separately heated inlet block, water circulating and purification system, detector cell and dc conductivity bridge. The sample is oxidized or reduced and reaction products form electrolytes when dissolved in the deionized water. Changes in conductivity between two platinum electrodes are measured by the dc bridge. (Figure 2 a)

E Recorder

The recorder system registers the response of the detector to sample components. In the case of ionization detectors, it is often necessary to employ an electrometer in order to amplify the small current changes.

Expensive integration and digital read-out equipment is also available to facilitate measurement of peak areas.

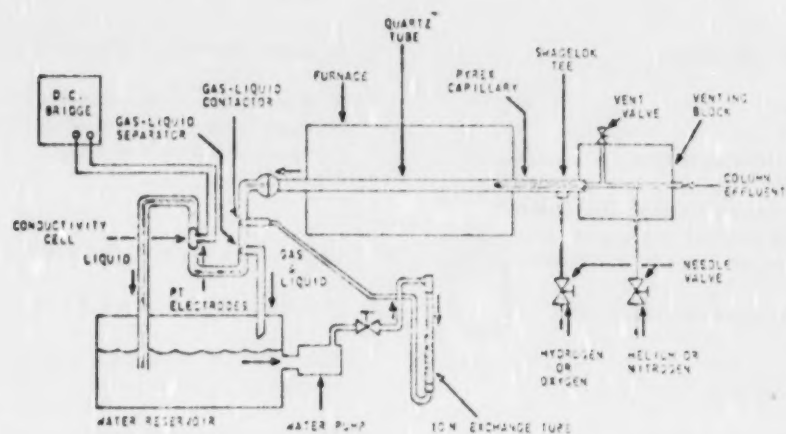


Figure 2 a. FLOW DIAGRAM OF ELECTROLYTIC CONDUCTIVITY DETECTOR
(Coulson Instruments Company)

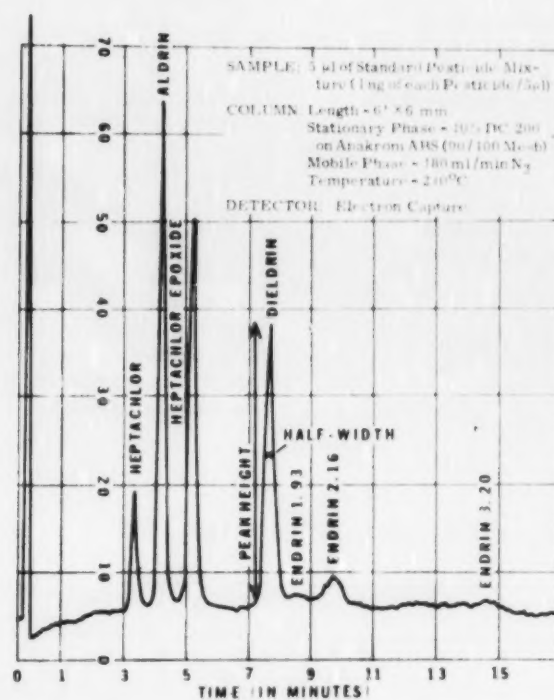


Figure 3. GAS CHROMATOGRAM OF PESTICIDE MIXTURE

III QUALITATIVE ANALYSIS

A Retention Time

The retention time of a sample component is defined as the time it takes for that component to travel through the column. There are a number of variables which affect the retention time of a compound.

- 1 Physical parameters of column operation
 - a Column length
 - b Column temperature
 - c Carrier gas flow rate
- 2 The nature and amount of stationary liquid itself

For a given set of column conditions, a specific compound will have a specific retention time (See Figure 3 and Table 5). Various column and detector combinations can be used to confirm identification.

B Retention Volume

Retention volume is defined as the total volume of gas required to move a component through the column.

$$\text{RETENTION VOLUME (R}_V\text{)} = \text{RETENTION TIME (R}_T\text{)} \times \text{FLOW RATE}$$

C Relative Retention Times and Volumes

It is possible to interpret data more easily by reporting retention data relative to a particular compound (e.g., aldrin as in Table 5).

IV QUANTITATIVE ANALYSIS

A Measurement of Peak Area

The quantity of sample component present is directly proportional to the area under its peak. (NOTE: This assumption can only be made if it has been previously determined that a linear response is obtained in the range under study.) The following are a few of the ways in which this area can be measured.

- 1 Planimeter
- 2 Triangulation
- 3 Peak height $\times$ half-width (see dieldrin peak in Figure 3)

$$\text{AREA} = \text{peak height} \times \text{peak half-width}$$

- 4 Disc integrator

B Measurement of Peak Height

With the electron capture detector it may be possible to use peak height for quantitative measurements where the following conditions are met.

Table 5. RETENTION DATA FOR FIGURE 3

Pesticide	Retention Time (R _T)	Relative Retention Time
Heptachlor	3.3 minutes	0.79
Aldrin	4.2	1.00
Heptachlor Epoxide	5.3	1.26
Dieldrin	7.7	1.84

- 1 A steady baseline is obtained.
- 2 Retention times can be reproduced from one injection to the next.

V SUMMARY

The basic components of a gas chromatograph have been described. Elementary aspects of quantitative and qualitative analysis are presented.

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CHARACTERISTICS OF THE FLAME IONIZATION DETECTOR

When gases burn in a flame and become sufficiently hot a small percentage of the molecules ionize and give rise to an electrical conductivity within the flame. When an organic molecule enters this flame it causes this conductivity to increase. This increased conductivity is measured and recorded. Thus, we have the basis for a very sensitive method for the detection of organic material. The credit for this observation is given to Harley, Nel, and Pretorius and to McWilliam and Dewar^(1, 2, 3)

The exact mechanism within the flame which is responsible for the conductivity has been the subject of considerable smoke and very little light and for this reason will not be discussed here. Interested parties will want to consider references 4, 5, 6, 7, and 8 for further information on this point. It should be mentioned however that the efficiency of the flame ionization detector (FID) is very small having an upper limit of approximately 0.01% and ranging down to 0.0001% for most common commercial detectors.

The electrical characteristics of the FID should be mentioned since they give us a clue as to its best mode of operation. In normal practice one usually puts 200 to 300 volts across the electrodes to collect the ions. The resistance of a hydrogen flame is of the order of 10^{-14} ohms while the background current is approximately 10^{-12} amperes and the signal current may run up to 10^{-7} amperes. The measurement of such small currents demands that we use a high impedance amplifier (electrometer) and some care in the design of the FID itself along with the associated circuitry.

SENSITIVITY AND LINEARITY

It should be kept in mind that the FID is a mass flow detector rather than a concentration detector. This means that the response of the flame is dependent on the number of grams

of carbon which enter the flame per second rather than the concentration of carbon in the carrier gas entering the flame. The limit of detection for this device is expressed in such terms as 1 in 10^{-9} molar, one part per billion, 2×10^{-13} gram atoms of carbon, or 5×10^{-12} grams of carbon per second. The linearity of the flame commences at detectability and runs to at least 0.5% while the dynamic range covers four to six orders of magnitude.

CALIBRATION

This device demands careful calibration and operation for good quantitative results as shown by the fact that the molar response for 2,2, 4-trimethylpentane is 30% greater than that for ethyl benzene and 15% greater than that for n-octane (all C_8 molecules).

One usually calibrates a FID by determining response factors for the compounds of interest. This response depends on geometry and operating conditions of the FID and is usually expressed in coulombs per gram of carbon or in coulombs per gram atom of carbon. In the ultimate analysis what we wish to know is the absolute or relative amount of material leaving the chromatographic column. This amount is the integral of the detector current with respect to time providing the detector signal and mass flow rate are linear over the range in question.

Figure 1 and Equation 1 illustrate what we are measuring.

$$I = r \times g \times t^{-1} \quad (1)$$

where:

I = amperes

r = coulombs/gram

g = grams of carbon

t = time in seconds

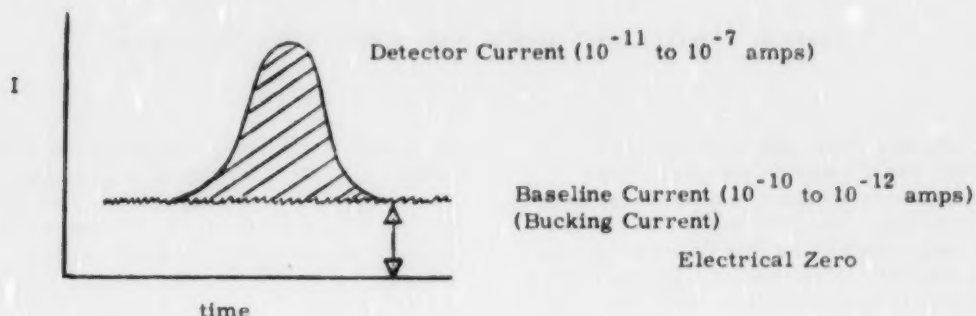


Figure 1

OPERATING PARAMETERS OF THE FID

In general the electrode geometry and polarizing voltage for the detector are determined by the manufacture and you have very little control over them. This is, however, an important point and should not be overlooked in the purchase of new equipment.

GASES USED

When using a FID and working in the low parts per billion region certain problems become acute. Foremost among these is the need for "clean" gases (hydrogen, air, nitrogen or helium). Using proper techniques⁽⁸⁾ it is possible to get sensitivity to the low parts per billion range.

For hydrogen: Use a palladium-silver diffusion purifier or a liquid nitrogen cold trap changed with Davison 912 silica gel.

For air: Air taken through a combustion furnace at 825°C and then over a drying agent such as Drierite.

For helium: Use a liquid nitrogen trap; still some residual methane gets through.

TYPICAL GAS FLOW RATES FOR FID

The values listed below are "average" values. For maximum sensitivity one must experimentally determine what the best flow rates are for each individual detector.

Scavenger gas: Either air or oxygen may be used at a flow rate of between 200 and 500 cc/minute depending on the geometry and flow patterns within the detector.

Fuel gas: Hydrogen is normally used at a rate given by:

$$H_2 = \frac{1}{3} (\text{carrier gas} + 10) \text{ cc/minute}$$

Carrier gas: Usually helium or nitrogen is used at a rate given by:

$$\text{Carrier gas} = 3(H_2 \text{ flow} - 10) \text{ cc/minute}$$

Many times this flow rate is determined by chromatographic conditions and it may be necessary to add "make up" gas between the end of the column and the flame detector.

OPTIMIZING FID PERFORMANCE

- 1) Dependence of ion current on polarizing voltage
- 2) Dependence of ion current on mass flow rate of sample
- 3) Dependence of ion current on scavenger gas rate
- 4) Dependence of ion current on fuel gas rate
- 5) Dependence of normalization factors on fuel gas rate

CONCLUSION

A great deal of information is available today on the design and operation of flame detectors. In order to do good quantitative work the operator of such a detector must take the time to understand its operation and limitations. Used with care this detector will give very good results - however, if used in an indiscriminate manner it will lead to results which may contain large errors. The type of work you do will depend on your understanding of this detector. (10)

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- This outline was prepared by W.E. Baitinger, Department of Chemistry, Purdue University, Lafayette, Indiana.

Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Water Analysis

QUANTITATION IN GAS CHROMATOGRAPHY

I INTRODUCTION

One of the factors which has led to gas chromatography's rapid development in the field of instrument analysis is the quantitative precision with which samples can be analyzed. There are several equally important factors involved in quantitative analysis. These are: Accurate sample introduction, constant operating parameters, accuracy of peak area measurement, sensitivity factors of individual compounds, linearity of detector and columns which give well resolved peaks.

II CALIBRATION PROCEDURE

A Peak Height and Peak Area

Quantitative work is based on peak height or area. Peak height measurement is more rapid than peak area; however, plots of peak height vs. sample size are more non-linear than corresponding plots for peak area. This is because peak heights and widths are frequently dependent on sample size and sample feed volume, however; total area is not. Peak heights are generally used if samples are less than $10\mu\text{g}$ for packed columns and $0.1\mu\text{g}$ for capillary columns.

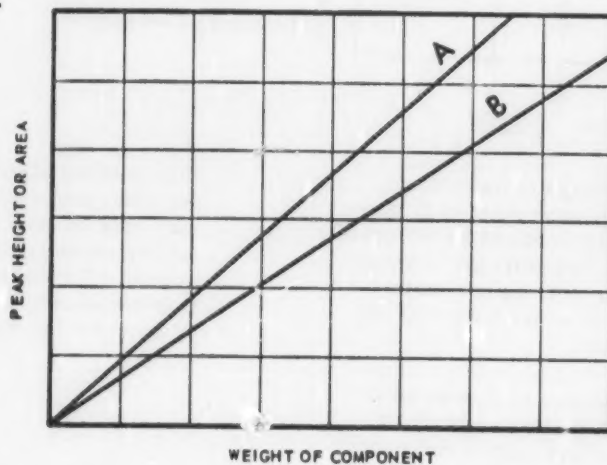
B Direct Calibration

Blends of components in question are prepared and chromatographed. The value of peak heights or areas are then plotted against the weight of sample injected. The unknown sample is then injected and its peak height or area is compared to that of the standard.

The main disadvantages of direct calibration are that the precise amount of sample injected must be known and that calibration is time consuming. Also, the sensitivity of the detector must remain constant from run to run and day to day in order to compare results with the calibration graph.

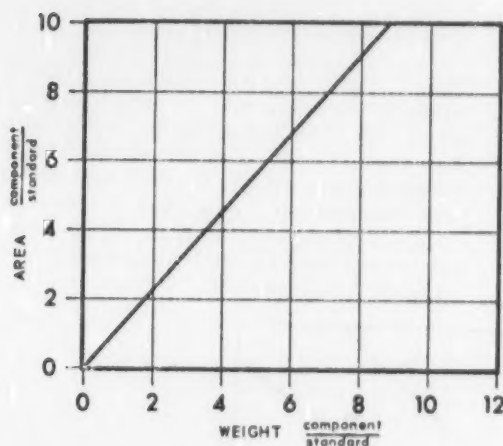
C Internal Standardization

This is a common procedure used in biomedical analyses, such as alcohol in blood, and steroids in serum and urine. Known weight ratios of the component in question and a standard (marker) are prepared and chromatographed. The component/standard area ratios obtained are then plotted vs. the component/standard weight ratios.



To the unknown, add a known volume concentration of the standard. This mixture is then chromatographed and the component/standard area ratios are determined. By determining the corresponding component/standard weight ratio from the graph, the unknown amount of the component can be determined.

e. g.: To the unknown, add 5 ml of a solution containing the standard whose concentration is 100 $\mu\text{g/ml}$. Upon chromatographing the mixture, the area ratio was found to be 8; therefore, the weight ratio is 7 (see graph). Knowing standard concentration to be 100 $\mu\text{g/ml}$, then the component concentration is $7 \times 100 \mu\text{g/ml}$. Since we added 5 ml of standard solution, then the total amount of the unknown is $5 \text{ ml} \times 700 \frac{\mu\text{g}}{\text{ml}} = 3500 \mu\text{g}$ or 3.5 mg.

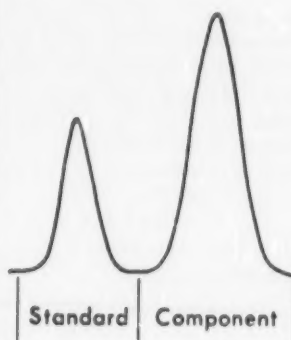
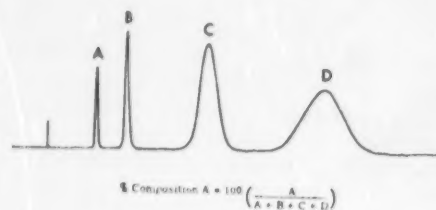


A particular advantage of using the internal standardization method is that the amount injected need not be accurately measured. Also, the detector response need not be known or remain constant since any change in sensitivity will not affect the area ratio.

The chief disadvantage of this method is the difficulty in finding a standard that does not interfere with a component in the sample. It is also time consuming.

D Internal Normalization

This is used when only approximate data are required. Assuming all the components have been eluted, the percent composition of a component within a mixture is its area divided by the sum of the areas.



This method also assumes that area percent equals weight percent. This may only be true when analyzing close boiling components of a homologous series. To obtain the weight percent, it is necessary to multiply each area by a correction factor.

III NORMALIZATION OF AREAS (CORRECTION FACTORS)

A Normalization for Thermal Conductivity Detectors

This procedure relies on the fact that each compound has a unique thermal conductivity cell response. For quantitative analysis, the thermal conductivity of the sample should be approximately a linear function of its composition in the range concerned. From the areas under each peak and the relative responses that are characteristic of each peak, it is possible to determine the quantity of each component in the sample.

Sample calculation:

COMPOUND	AREA	RELATIVE T.C. RESPONSE PER MOLE (2)
Ethanol	5.0	72
Heptane	9.0	143
Benzene	4.0	100
Ethyl Acetate	7.0	111

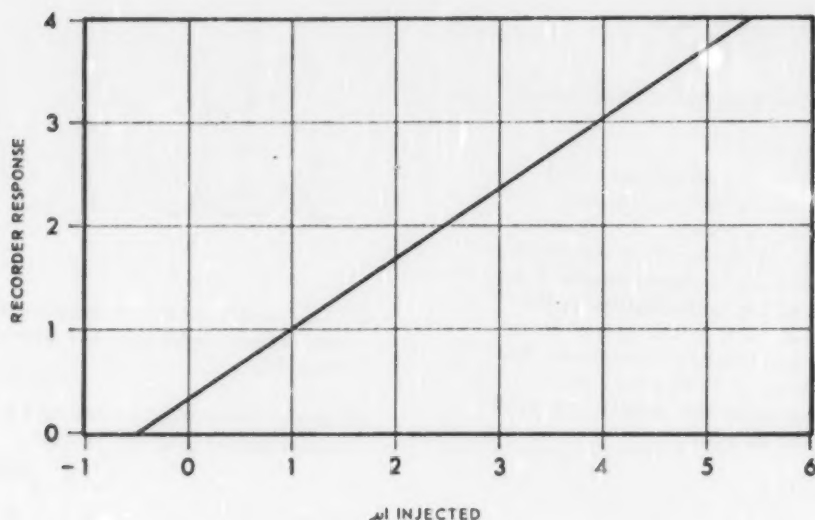
			NORMALIZE		MOLE %
Ethanol	$\frac{5.0}{72} = 0.070$		$\frac{0.070}{0.236}$	=	29.6
Heptane	$\frac{9.0}{143} = 0.063$		$\frac{0.063}{0.236}$	=	26.7
Benzene	$\frac{4.0}{100} = 0.040$		$\frac{0.040}{0.236}$	=	17.0
Ethyl Acetate	$\frac{7.0}{111} = 0.063$		$\frac{0.063}{0.236}$	=	26.7
TOTAL			0.236		100.0%



- The weight percent is calculated and the known densities are used to convert percent.
- Inject several samples and plot peak area (or height) vs. volume injected.

B Normalization for Mass Detectors

- A standard solution of compounds a, b, c, d, and e in a solvent is prepared.



- 4 Note that intercept on "X" axis corresponds to an injection error of 0.5 μ l. Therefore, actual amount injected = uncorrected + 0.5 μ l. For 2.5 μ l (corr.) injected, the density values are used to determine the actual weight W. injected.
- 5 The corresponding area of each peak is determined and the response in $\text{cm}^2/\mu\text{g}$ is determined.
- 6 Using compound "a" as standard (correction factor = 1), the corresponding correction factors are determined. See the Table on Page 8-6.

IV STATISTICAL TREATMENT OF DATA

A Accuracy and Precision Data (Reproducibility)(3, 4)

Accuracy is a measurement of the difference between the true value and the determined values. In those cases in which the true value is not known, it is necessary to express the exactness of a measurement in another way. This may be done by obtaining the average of a number of measurements and finding the

difference (deviation) of each value from the average value. The magnitude of deviation is a measure of the precision of measurement.

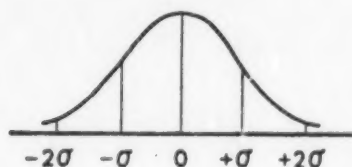
It is seen that accuracy expresses the correctness of a measurement, whereas precision expresses the reproducibility of a measurement.

The accuracy and reproducibility achieved in gas chromatography depend on many things; among them, the correct choice of column, temperature, flow rate, sample size, detector, and injection system. Highest performance demands considerable understanding of the chromatographic process and of the effects of change in a large number of variables. However, even with ill-designed apparatus, analyses can usually be conducted with an accuracy better than + 10 percent per component. When precautions are taken, an accuracy and reproducibility of about + 1 percent per component is easily attainable.

B Errors

Errors fall into two classes. Determinate errors are those whose cause and magnitude can be determined. (Errors in method,

apparatus, operator, etc.) Indeterminate errors are random errors which cannot be eliminated. The distribution of indeterminate errors follows the normal probability law as shown in the error curve. This curve shows that positive and negative deviations are equally probable and that small deviations occur much more frequently than large ones.



σ = Standard Deviation

$\pm\sigma$ = 68%

$\pm 2\sigma$ = 95%

V MEASUREMENT OF AREAS

A Methods

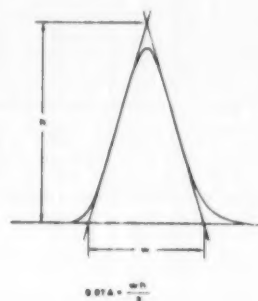
- 1 Cutting and weighing is a fairly accurate but tedious procedure. Besides the errors that may arise from thickness and moisture content of the paper, the chromatogram is destroyed. It is not satisfactory for estimating areas of overlapping peaks.
- 2 The planimeter is a device whose acquisition is rarely justified. In addition to being very fatiguing, it is necessary to acquire considerable practice in its manipulation. The sensitivity of a normal planimeter is usually 10 mm^2 , which is in many cases insufficient for analytical purposes. (5)

3 Integrators

- a Mechanical integrators are exemplified by the Disc Integrator. This is a device which is attached to most strip chart recorders. The integrator pen tracing is displayed at the bottom of the chromatogram and the pen speed is proportional to the displacement of the recorder pen from baseline. (See triplicate runs of the nonanodecane mixture.) This is a frequently used, accurate device.
- b Electronic integrators are devices which automatically print cumulative integrals of peak area. In addition to offering outstanding precision, electronic integrators automate chromatographic operations. This eliminates the time consuming necessity of the chemist keeping a watchful eye on the operation. Its biggest disadvantage is its cost.

4 Triangulation

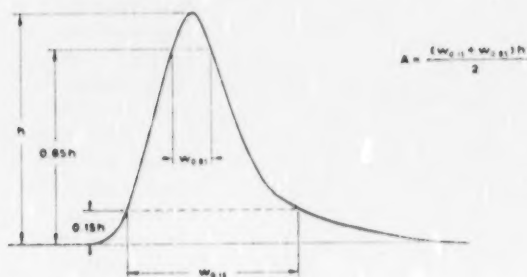
a Gaussian peaks



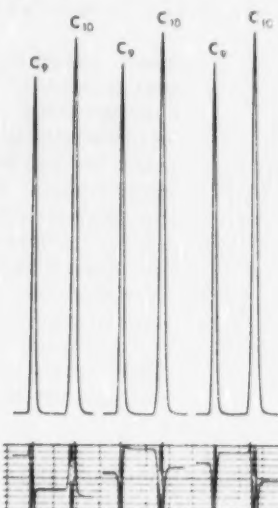
SAMPLE	WT. %	DENSITY gm/cm ³	VOL. %	VOL. INJECTED, μ l		W WT. INJECTED μ g	A AREA cm ²	cm ² / μ g	F CORRECTION FACTOR
				uncorr.	corr. (actual)				
Solvent	99.79	.88	99.781	1.99562	2.49453	2195.19	--	--	--
a*	.02	0.50	.035	.00070	.00087	.435	4.0	9.19	1.00
b	.03	0.75	.035	.00070	.00087	.653	6.5	9.95	1.08
c	.04	0.90	.0387	.00077	.00096	.864	7.6	8.79	.96
d	.04	0.90	.0387	.00077	.00096	.864	8.1	9.38	1.02
e	.08	1.00	.0704	.00141	.00176	1.760	15.0	8.52	.93
Total	100.00		100.00	2.00	2.50	2199.766			

*Correction factors calculated relative to "a" as standard.

b Tailing or leading peaks, trapezoidal construction, *



Triplicate analysis of nonane-decane mixture



B Comparison of Integration Methods

Using several of the above methods on the triplicate runs of the nonanodecane mixture, the following table was obtained:

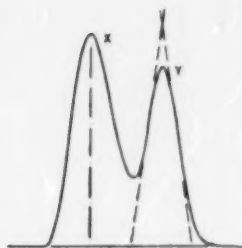
COMPARISON OF INTEGRATION METHODS

	#1	#2	#3	Avg.	σ_{abs}	σ_{rel}
MODEL 471 DIGITAL INTEGRATOR						
n-Nonane	39.156	39.150	39.193	39.166	0.0716	0.184%
n-Decane	60.844	60.850	60.807	60.834	0.0227	0.037%
DISC INTEGRATOR						
n-Nonane	39.33	38.97	38.91	39.07	0.22	0.56%
n-Decane	60.67	61.03	61.09	60.93	0.23	0.38%
TRIANGULATION						
n-Nonane	40.77	40.68	40.07	40.51	0.38	0.94%
n-Decane	59.23	59.32	59.93	59.49	0.39	0.66%
WEIGHING PAPER						
n-Nonane	42.58	41.73	42.83	42.38	0.58	1.37%
n-Decane	57.42	58.27	57.17	57.62	0.58	1.01%

Summary: Electronic digital integration is the most precise quantitation method; weighing paper is the least precise. Based upon this and other data in this publication, the electronic integrator give 2 to 5 times more precision than Disc Integration, 4 to 10 times more precision than triangulation, and 7 to 25 times more precision than cutting and weighing paper.

C Measurement of Overlapping Peaks

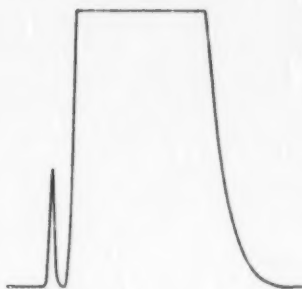
1 Components present in equal amounts.



Since broken line tangent to peak y intersects baseline after peak x has reached its maximum, then peak heights may be used. If broken line tangent to peak y intersects baseline before peak x has reached its maximum, then various known mixtures of x and y are prepared, chromatographed, and compared to the unknown x and y.

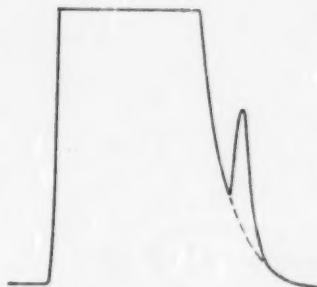
2 Trace analysis (less than 20 ppm)

a Trace component eluting before major peak.



In any trace analysis, one should choose a column and conditions which will allow the minor peak to elute before the major peak. This is easier to quantitate, and generally, peak height methods are used.

b Trace component on tail of major peak.



Assuming one has tried all conceivable columns and conditions, extend baseline as a continuation of the solvent peak and determine area. Compare this area with a synthetic mixture. Also, one can "spike" the sample with known amount of the trace component and compare area before and after.

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This outline was prepared by E. J. Bonelli, Applications Manager, Varian Aerograph Company, Walnut Creek, California.

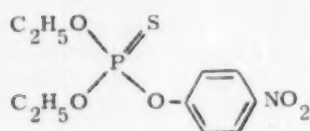
Descriptors: Chemical Analysis, Chromatography, Gas Chromatography, Instrumentation, Measurement, Separation Techniques, Water Analysis

SPECIAL TECHNIQUES REQUIRED FOR THE ANALYSIS OF PHOSPHORUS AND HERBICIDAL COMPOUNDS

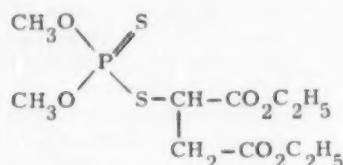
A Organic Phosphate Insecticides

- 1 Compounds such as, parathion, malathion, EPN, ethion, ronnel.

a Parathion

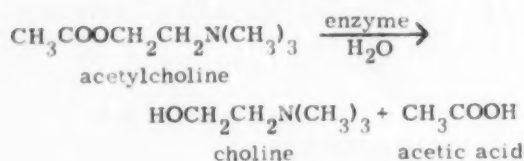


b Malathion



2 Cholinesterase methods

- a When acetylcholine is incubated with cholinesterase, acetic acid and choline are produced according to the following equation:



- b The amount of acetic acid formed can be measured manometrically, titrimetrically or potentiometrically.
- c When organic phosphates are present, the enzyme is inhibited and the amount of acetic acid formed is reduced in proportion to the degree of inhibition.

3 Non-specific gas chromatographic detectors

a Electron capture

- 1) Sensitivity not as great as for chlorinated hydrocarbons but better than with flame ionization detector.

b Hydrogen flame ionization detector

- 1) When an organic compound is burned in a hydrogen flame, the conductivity is increased.
- 2) The response is believed to be directly related to the thermally generated carbon ions in the burned compounds, and hence, response varies with the number of carbon atoms present.

4 Specific gas chromatographic detectors

a Sodium thermionic detector

- 1) Same as the conventional flame detector except that a coat of a sodium salt has been fused onto the electrode.
- 2) The response of this detector to an organic compound containing 10 carbon atoms and 1 phosphorus atom is about 600 times greater than if it contained no phosphorus.
- 3) The response to halogen ions is much less and the carbon response is unchanged.

b Stacked thermionic flame detector

- 1) Bottom flame is a normal flame detector, while top flame has a sodium grid.

2) Response to halides and phosphates in the upper flame is of the order of 100,000 - 200,000 to 1.

3) Air-hydrogen gas mixture gives enhanced response preferentially for phosphates, while oxygen-hydrogen mixtures produce enhanced response for halides preferentially.

c Flame photometric detector

In this detector, the effluent coming from the column is mixed with an air/oxygen mixture and burned in a flame that is surrounded by a cover of hydrogen. The flame is seated within a high-walled cup. Fragments of the organic species in the sample are excited to higher energy states in the reducing atmosphere of the flame. The area above the cup is viewed by a standard photomultiplier tube through a narrow bandpass filter of the wavelength of the desired element.

An electrode located above the flame provides a simultaneous flame ionization output. When a single sample is injected, a standard flame ionization trace as well as that for sulfur or phosphorus components is recorded. When dual filters and dual photomultiplier versions are used, a simultaneous readout of sulfur, phosphorus and flame ionization response may be obtained.

The choice of maximum transmittance at 526 m μ for phosphorus HPO species in a hydrogen flame and of 394 m μ for sulfur S₂ emissions allows the detection of P or S in organic compounds with only minimum interference. The present detection limit of the flame photometric detector is 10 pg of phosphorus and 40 pg of sulfur.

Positive confirmation regarding the presence of phosphorus or sulfur compounds can be made.

d Microcoulometric detector

1) In the conventional microcoulometric gas chromatograph, the column effluent is oxidized with molecular oxygen to yield CO₂, H₂O, HCl and SO₂. HCl can then be measured specifically with a titration cell equipped with silver electrodes, and SO₂ with an I₂/I-cell. This method is not applicable to phosphates.

2) If the column effluent is reduced with molecular hydrogen at 950°C, phosphates are converted to PH₃, organically bound sulfur to H₂S and organically bound chlorine to HCl. All three of these gases precipitate silver ion and can be measured with a microcoulometric titration cell.

3) If a short subtraction tube containing Al₂O₃ is inserted between the exit line of the reduction tube and the inlet of the titration cell, H₂S and HCl are subtracted quantitatively. PH₃ passes through this packing unchanged and thus can be measured with absolute specificity.

5 Types of columns used for gas chromatography

a Same as for chlorinated hydrocarbons, except that glass columns are a must.

b Some suggested columns are:

1) 10% DC 200 on Anakrom ABS

2) 3.5% SE-30 on Chromoport XXX

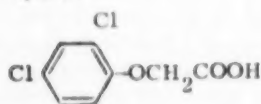
6 Clean-up procedures

- a In general for the fat soluble organic phosphates, the same as for the chlorinated hydrocarbons.

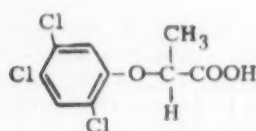
B Phenoxyalkanoic Acid Herbicides and Their Esters

- 1 Compounds such as, 2,4-D, 2,4,5-T, silvex, and their esters.

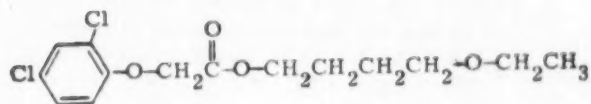
- a 2,4-D



- b Silvex



- c Butoxy-ethanol ester of 2,4-D



2 Esterification techniques for free acids

- a The esters of 2,4-D, 2,4,5-T and silvex can be chromatographed on and separated by some of the usual type of gas chromatographic columns but the free acids cannot be unless specific types of columns are used and even these only with difficulty.

- 1) Methylation with diazomethane is rapid, convenient, and gives excellent yields, however, the reagent is extremely poisonous and has poor storage life even when kept in the cold.

- 2) Methanol-boron trifluoride mixture methylates rapidly but heat must be applied. In general, the yields are lower than with diazomethane but this varies with the compound. The reagent has good storage life and is relatively safe to use.

3 Clean-up procedures

- a Most of the esters can be hydrolyzed by treatment with alkali; namely, shaking a homogenate with dilute alkali for several hours. The sample is then analyzed for total free acid.

- b Alternately, the esters and free acids may be separated and analyzed separately.

- 1) The sample is acidified and both the free acid and ester extracted with ether, benzene or chloroform.

- 2) The ether is in turn extracted with a buffer solution. The free acid will be partitioned into the buffer while the esters will remain in the ether solution.

- 3) The buffer solution is acidified and the 2,4-D extracted with chloroform. The chloroform is evaporated, the residue taken up in ether and methylated with the methanol BF₃ mixture.

- 4) The ether solution remaining from the initial extract contains the esters and is further cleaned-up by chromatography on alumina and florisil.

- 5) For products of low moisture content such as dried beans, grains, seeds or nuts, the sample must be partially rehydrated before adding solvent for extraction; otherwise the recovery of 2,4-D is poor.

- 6) Potatoes may require a special cleanup to remove interfering natural occurring halogen-containing compounds.
- 4 Type of column and detector
 - a 20 percent Dow 11 silicone grease on acid-washed Chromosorb P.
 - b Can be detected satisfactorily by either electron capture or microcoulometer.
- 5 Colorimetric procedure
 - a After cleanup as above, the 2, 4-D can be measured by development of a wine-purple color with chromotropic acid.

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INFRARED INSTRUMENTATION

I INTRODUCTION

In infrared work the spectroscopist is using a spectral region which is not visible to the human eye. The types of visual comparators commonly used in the laboratory cannot be applied to measurements in the IR. Thus the analyst is completely dependent upon an electrical system for his evaluations.

Because of the specific nature of the IR spectra of most substances, the full recorded spectrum, with its characteristic peaks is generally used. The complexity of the IR spectrum rules out the possibility of manual plotting of absorption curves. Sensitive recorders, having rapid response, are required.

The region generally examined extends from 2.0 - 15 μ (Fig. 1). Some research instruments are capable of scanning a much wider range, from 1.0 - 40 μ . In this portion of the spectrum energy levels are low and strong absorption bands are found.

As in all analytical spectroscopy, the measurement system involves a source of radiant energy, an arrangement for placing the sample in the incident beam, and a detector or receptor for measuring the changes in energy caused by absorption.

II SOURCE OF RADIANT ENERGY

A Nernst Glower

This type of element consists of a mixture of rare earth oxides bonded into a small rod. When electrically heated this source gives off infrared energy of the desired wavelengths.

B Globar

In many newer instruments a rod of bonded silicon carbide is used. Like the glower, the energy emitted is in the desired region of the spectrum.

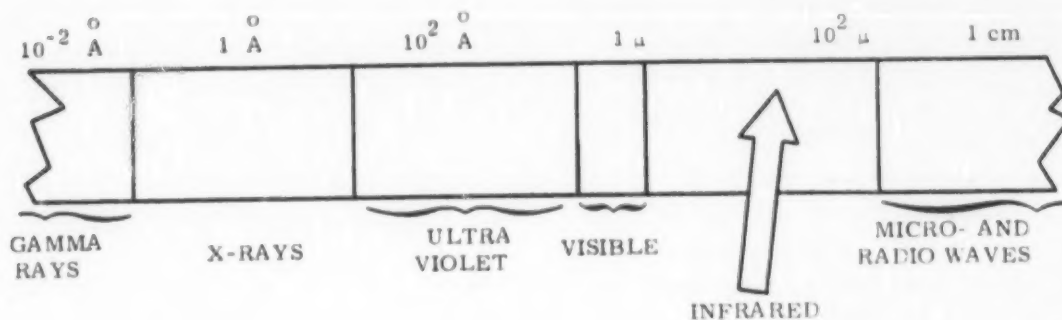


Figure 1 - ELECTROMAGNETIC SPECTRUM

C Nichrome Wire

A few IR instruments use an electrically heated nichrome wire as a source.

D Monochromator

In order to provide a spectrum for study the IR instrument must disperse the energy from the source into small increments and selectively direct these wavelength components through the sample. These functions are accomplished by the monochromator. Since the glass optics used in visual range instruments will not transmit infrared energy, a prism of another material must be used. (Fig. 2) shows the optical materials commonly used, with the effective range of each.

In order to maintain constant energy level with changing wavelength, the slit is "programmed". That is, the slit changes as the wavelength increases, compensating for the loss of energy at the longer wavelengths.

III CELL ASSEMBLY AND CHARACTERISTICS

A Geometry

In most photometric instruments the radiant energy reaches the sample after being dispersed into selected wavelengths. In IR spectrophotometers it is possible to place the sample in the undispersed beam and then pass the beam through a suitable monochromator to the detector. In the double beam instrument shown in Fig. 3 the energy from the source is split into two equal beams. One beam passes through the sample, the other is unchanged and thus becomes a reference. The recorder indicates the difference in energy between the two beams; that difference is the result of absorption of radiant energy by the sample.

B Cell Characteristics

1 Materials

The factors which govern the selection of optical materials in the monochromator

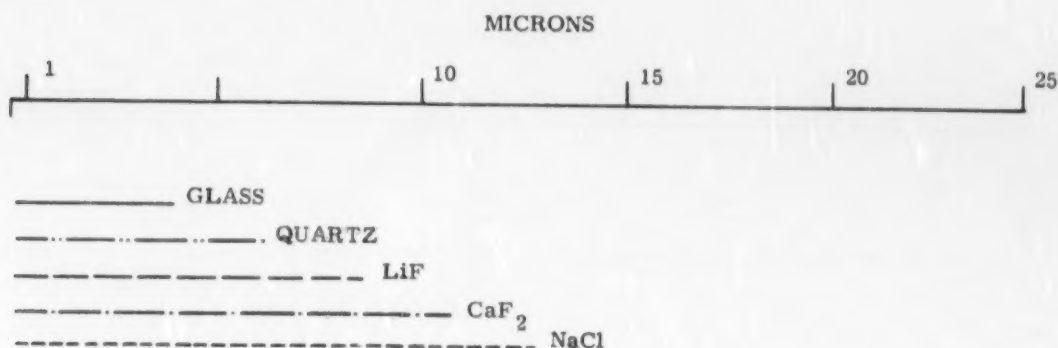
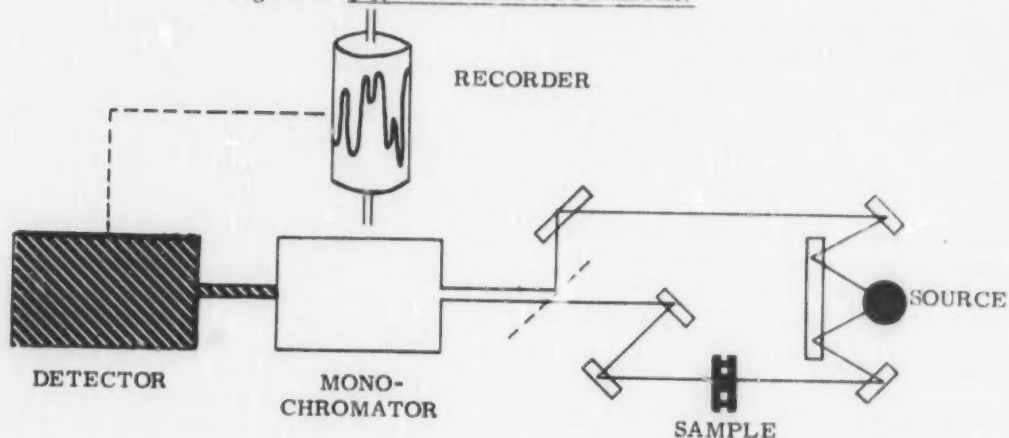


Figure 2 - OPTICAL MATERIALS

Figure 3 - I-R SPECTROPHOTOMETER



also apply to cell materials. See Fig. 2 Sodium chloride windows are used for most IR work. Because of the solubility of NaCl in water, AgCl windows must be used for aqueous samples. In fact, the hygroscopic nature of the NaCl requires constant care to prevent fogging by atmospheric water vapor. NaCl cells are always stored in a dessicator when not in use.

Fig. 4 shows one type of IR cell. The windows are clamped in place with a lead or Teflon spacer between, to provide proper light path.

2 Light Path

Because of the strong absorption bands in the IR region, the light path is very short. The most common cell paths are 0.025, 0.10 and 1.0 mm. With certain organic compounds a satisfactory spectrum can be obtained with only 0.01 mm sample distance.

When working with volatile solvents a sealed cell is required. The cell is

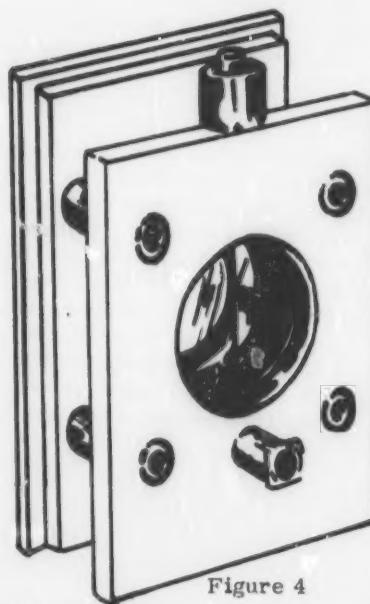


Figure 4

FIXED THICKNESS ABSORPTION CELL
(Courtesy Perkin - Elmer Corporation)

filled by means of a hypodermic syringe. After filling, the ports are sealed with Telfon plugs.

C Special Cells

1 Smears

When non-volatile materials are being examined, the sample can be smeared directly onto a cell window, with or without a covering window.

2 KBr Technique

Crystalline solids cannot be examined directly, due to light scattering by the crystals. A technique has been developed using potassium bromide, whereby a solid can be mixed with KBr and compressed into a very thin sheet. Since the KBr is transparent in the IR, these discs permit the examination of the solid sample. Preparation of the KBr discs is difficult but opens the way to the examination of a wider range of organic and inorganic materials.

3 Gas Cells

Because of the high sensitivity of the IR Spectrophotometer, it is possible to obtain the spectrum of gases as well as liquids and solids. Obviously the molecular density of gases necessitates a much longer light path. Special gas cells have been developed with path lengths from 5 cm to 10 meters. The 5 cm cells have a single pass light path, while the longer paths are obtained by multiple passes, as shown in Fig. 5.

IV DETECTOR

A Theoretical

Radiant energy in the IR region is actually heat radiation. The absorption of energy at various wavelengths is the absorption of heat. Thus an infrared detector is a heat-measuring device. The most commonly used detectors are the thermocouple and the Bolometer. Although these measure the ambient temperature as well as the signal, it is only necessary to measure the difference between the sample beam and the reference beam. Further, since radiant energy is being measured, small temperature differences in the sample have no effect.

B Thermocouple

The bimetallic junction of the thermocouple is capable of measuring energy changes over the whole IR range. Because a very rapid response is required, special thermocouples have been designed for IR applications.

C Bolometer

Certain metals have the property of high thermal resistivity. That is, the electrical resistance of the metal changes greatly with change in temperature. The bolometer consists of an element of such a metal mounted as a target in the IR beam. Changes in resistance, produced by energy fluctuations, are converted to electrical impulses and fed to the amplifier circuit.

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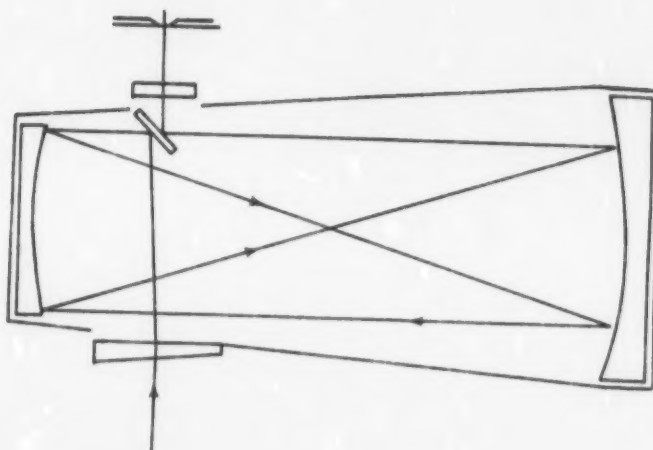
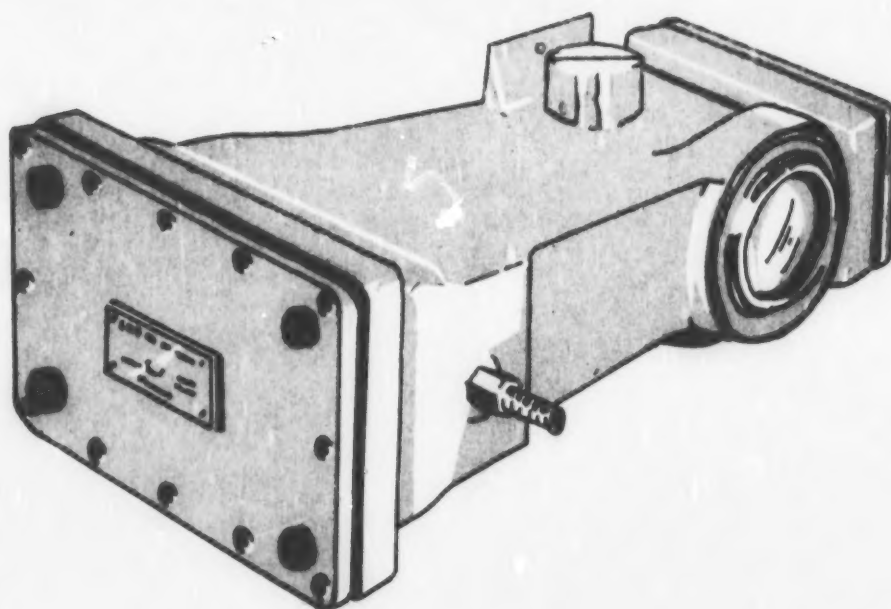


Figure 5 - ONE METER GAS ABSORPTION CELL
(Courtesy Perkin - Elmer Corporation)

PRINCIPLES OF INFRARED SPECTROSCOPY

I GENERAL

The term "infrared spectroscopy" refers to the technique of spectroscopic measurement in the infrared region of the electromagnetic spectrum. This region starts at a wavelength of approximately 760 μ m and ends at about 1000 μ m. Wavelengths shorter than 760 μ m are in the region of visible light, while wavelengths longer than 1000 μ m (1 mm) are in the microwave region.

For practical reasons, the infrared region is divided into three ranges: The near infrared (NIR) range extends from 760 μ m to 5 μ m, the mid-infrared from 5 to 25 μ m, and the far-infrared extends from 25 μ m to 1 mm. Other definitions refer to the molecular vibrations encountered in these ranges. Overtone, fundamental, and rotational vibrations succeed each other with increasing wavelength (Figure 1).

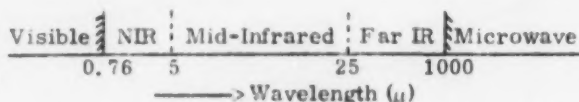


Figure 1

In the infrared region, wavelengths (λ) are commonly measured in microns (μ) down to 1 μ , and below 1 μ they are measured in millimicrons ($m\mu$). Another unit, the wave-number (γ), which is closely related to frequency (f), is also used because frequency is a more fundamental unit than wavelength. Whereas the wavelength varies inversely with the velocity of monochromatic light traveling through a medium, the frequency remains constant no matter what the medium. These units are related as follows:

$$v = f\lambda, \text{ where } v = \text{the velocity of light}$$

$$\gamma = \frac{1}{\lambda} \text{ in waves per cm, or cm}^{-1}$$

$$f = v\gamma$$

$$1\mu = 10^{-4} \text{ cm} = 10^3 m\mu = 10^4 \text{ \AA}$$

$$\gamma (\text{in cm}^{-1}) = \frac{10^4}{\lambda (\text{in } \mu)}, \quad \lambda = \frac{10^4}{\gamma (\text{in cm}^{-1})}$$

Infrared absorption spectroscopy is based on the analysis of the spectrum from a high intensity infrared source after the emitted radiation has passed through a sample. By measuring the spectrum thus obtained, it can be seen (Figure 2) that part of the original radiation has been reduced in intensity after having left the sample; the amount varies with wavelength. The ratio of the intensities of the radiation measured before and after passing through the sample and plotted against wavelength gives a curve representing the absorption spectrum of the respective substance.

No two substances have yet been found whose infrared spectra are identical in all details. For this reason infrared spectra are excellently suited for identifying substances. Furthermore, knowledge of the theoretical background of infrared spectroscopy enables one to obtain information on the chemical structure and molecular geometry of a given substance.

II INSTRUMENT DESIGN

In its simplest form, a spectrometer consists of a source, S (Figure 3), a monochromator section to separate the various wavelengths, and some means of detecting D, and displaying R, the amount of energy emanating from the exit slit of the monochromator.

Radiation, comprising many wavelengths, is emitted by the source and enters the entrance slit, S_1 , of the monochromator. Energy from S_1 enters the prism P, and is dispersed into component wavelengths, which fall across the plane of the exit slit, S_2 . By rotating the prism, we can select the wavelength of narrow band of wavelengths that passes through S_2 to the detector, D. An indicator or recorder provides an indication proportional to the energy seen by the detector. This simple arrangement can be used to examine the fundamental limitations of spectrometers and their ramifications.

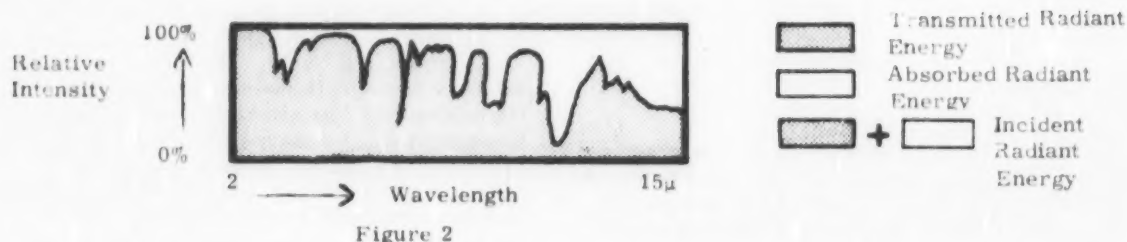


Figure 2

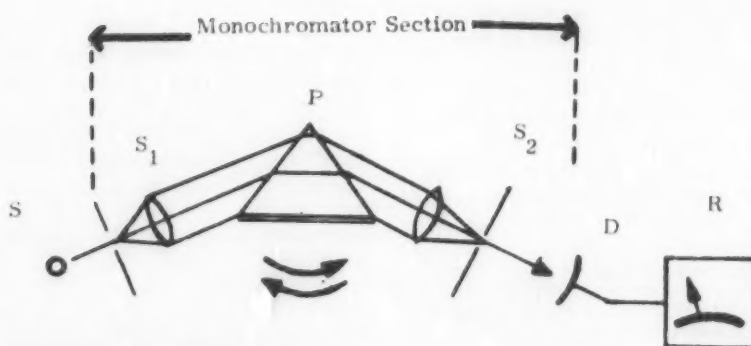


Figure 3

The double-beam infrared spectrophotometer provides a continuous record of the infrared transmittance of absorption of a sample as a function of the wavelength of the incident radiation. This is done by mechanically linking the recorder drum to the Littrow mirror. Since the angular position of the Littrow in reference to the prism face is directly related to the wavelength of radiation that appears at the thermocouple, the drum position will also be related to wavelength. A chart pre-plotted with wavelength of frequency of the ordinate and transmittance or absorbance of the abscissa is placed around the drum. As the Littrow assembly scans through the spectrum (2 - 15 microns), the differences of the two beam intensities at the photometer (caused by the spectral characteristics of the sample) are simultaneously recorded on the chart by a pen.

As shown in Figure 4, a scanning motor drives both the drum and the Littrow in the monochromator. The drum position always corresponds exactly with the wavelength of radiation under observation. Not shown (for simplicity) in Figure 4 is the linkage between the wavelength scanning mechanism and the monochromator entrance and exit slits, whose widths must be programmed simultaneously for constant energy at the detector.

The signals that drive the pen and indicate the transmission or absorbance of a particular sample are developed as follows: Infrared energy produced by a Nernst source is divided into two beams. One beam passed through the sample cell, which absorbs certain characteristic wavelengths. The other serves as a reference. The two beams

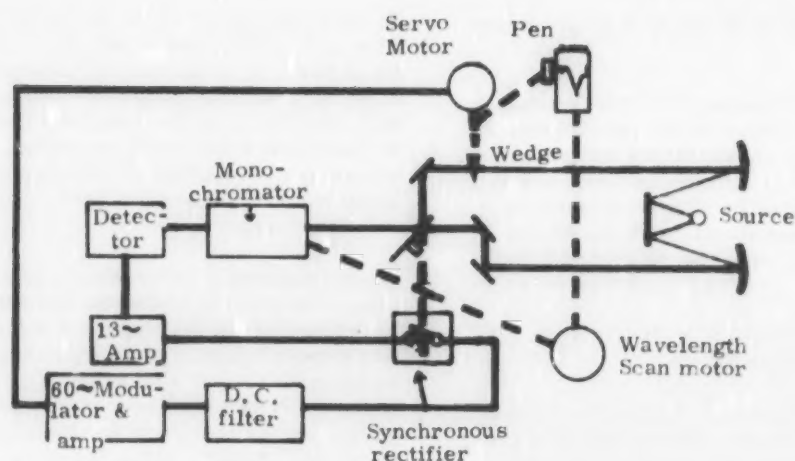


Figure 4

are recombined by the 13 cps chopped (a rotating semi-circular sector mirror) into a single beam consisting of alternate pulses of sample and reference beam energy. The monochromator disperses the combined radiation into its constituent wavelengths and allows a certain band of frequencies to emerge and fall on the thermocouple. If the energy in the sample and reference beams is equal, the alternate pulses of energy arriving at the thermocouple are equal. The result is that the thermocouple produces a d-c voltage which is not amplified by the a-c amplifying system. When, at the characteristic wavelengths of the sample, the intensity of the sample beam is reduced by **absorption**, a 13 cycle/sec. error signal is developed by the thermocouple whose amplitude is proportional to the difference in intensity of the two beams.

The following sequence now takes place: The 13 cycle error signal is amplified by a preamplifier that has a voltage gain of about 300. It is further amplified by a 13 cycle amplifier, synchronously rectified by mechanical breakers and filtered to obtain a smooth d-c signal, and remodulated to obtain a 60 cycle

signal. The 60 cycle signal is then amplified so that it can be used to drive the two phase pen servomotor. The shaft of the pen servomotor is linked to the wedge and the recorder pen. As the shaft rotates, the wedge moves in a direction to nullify the difference between the sample and reference beams (the error signal), and the pen records the direction and amount of movement caused by the error signal.

The method of nullifying an error signal just described is called a closed loop servo system. The functioning of a closed loop servo system is to detect an error signal, amplify it, and then nullify it to zero.

The inherent characteristics of a double-beam spectrophotometer with a closed servo loop that utilizes a radiation null are immediately obvious when compared with a single-beam (open loop) instrument. Since the "loop" is closed through the electronic system, gain changes do not show on the chart as a gain change would on a single-beam instrument. Double-beam instruments are theoretically free from drift or changes in I_0 or signal sensitivity caused by small changes in source

emissivity that normally affect a single-beam instrument.

Because of its advantages, the double-beam instrument is preferred by the spectroscopist who does a variety of laboratory analyses. He can also perform differential analyses to nullify errors caused by sample impurities (by inserting a second cell, with the known sample impurities, into the reference beam).

III WINDOW MATERIALS, CELLS, AND SAMPLING TECHNIQUES

Table 1 summarizes some of the more important physical properties of materials which transmit infrared radiation and would be usable in infrared sample cells.

The substances that can be analyzed by infrared absorption spectrometry exist in almost every conceivable form. They may be solids, liquids, or gases; hard or soft; concentrated or dilute solutions; under pressure or in a vacuum. However they exist, means must be provided to permit their analysis in the infrared spectrometers described earlier. It is not surprising, therefore, that a wide variety of sample accessories is available to the analyst.

It will be helpful in considering the accessories discussed to keep in mind Beer's law, which relates the absorption of radiation to the physical characteristics of the sample and the amount of sample present in the spectrometer beam:

$A = Kcl$;

where: A = optical density or absorbance (the quantity to be measured);

K = the extinction coefficient of the sample material (a quantity which is characteristic of the sample material, only);

c = the sample concentration (may be controlled by the analyst);

l = the length of that portion of the sample beam that passes through the sample (may be controlled by the analyst).

The aim in selecting any sample accessory must be to position sufficient sample in the sample beam to allow the extinction coefficient,

K , to measurably affect the sample beam intensity. This means that the sample must be neither too thick nor too concentrated. If either condition exists the sample beam will, in effect, be blocked, and there will be no radiation intensity to measure. Also, if the sample is too thin or too dispersed, the absorption of the sample beam will be too small to measure.

The demountable cell consists of a lead spacer sandwiched between two windows of an infrared transmitting material such as NaCl, KBr, CaF₂, etc. (depending upon the wavelength range to be scanned), all held together by two stainless steel plates with apertures for transmitting the incident radiation. Sample is placed in the cavity formed between the windows by the spacer, the thickness of the spacer determining the length of the optical path through the sample. Several thicknesses of spacer are available including 0.025, 0.05, 0.10, 0.20, 0.50, 1.0 and 3.0 mm.

The demountable cell is useful for qualitative analysis of sample materials such as non-volatile liquids, viscose substances, mulls, and solid films which can be cast onto one of the windows. The cell is not intended for quantitative analysis where an accurate knowledge of the cell thickness and optically flat, parallel windows are essential. For such purposes the sealed cell, which closely resembles the demountable cell, should be used. Its thickness is carefully calculated and therefore the choice for quantitative analysis.

Special Sampling Techniques

A Smears:

When non-volatile materials are being examined, the sample can be smeared directly onto a cell window, with or without a covering window.

B KBr Technique:

Crystalline solids cannot be examined directly, due to light scattering by the crystals. A technique has been developed

TABLE 1
PROPERTIES OF INFRARED TRANSMITTING MATERIALS

	Long Wavelength Transmission Limit (microns)	Index of Refraction (n_D)	Cold Water Solubility (gms/100ml)	Soluble in	Maximum Size	Description of material
Arsenic Trisulfide Glass	12	2.59	0.000	Alkali	Limited only by design considera- tions	As ₂ S ₃ ; Homogeneous, red glass. Stable, Non- toxic.
Silver Chloride	23	2.07	0.000	NH ₄ OH NO ₂ S ₂ O ₃ KCN	4" Dia. Thin rolled sheets approx- imately 20" Dia.	AgCl; Colorless, cubic crystal. Isotropic. No cleavage.
Potassium Bromide	27	1.55	53.48 00°C	Alcohol Glycerine	7.5" Dia.	KBr; Colorless, cubic crystal. Hygroscopic. Soft, easily scratched.
Sodium Chloride	15	1.54	3.57 00°C	Glycerine	7.5" Dia.	NaCl; Colorless, cubic crystal. Hygroscopic. Scratches easily.
Sapphire	5-5	1.77	0.000	---	5" Dia.	Al ₂ O ₃ ; Hexagonal crystal. No cleavage. Does not scratch readily.
Lithium Fluoride	5	1.39	0.27 180°C	Acid	5" Dia.	LiF; Colorless cubic crystal. Cleaves. Scratches easily.
Calcium Fluoride	9	1.43	0.002 180°C	NH ₄ Salts Solution	6" Dia.	CaF ₂ ; Colorless, cubic crystal. Cleaves. Scratches easily.
KRS-5	36	2.63	0.02 00°C	Alcohol, HNO ₃ Aqua Regia	5" Dia.	KRS-5; Rad, cubic crystal. No cleavage. Strains easily.
Fused Quartz	4	1.43	0.000	HF	Limited only by practical optical homogeneity.	SiO ₂ ; Good mechanical and thermal properties. Isotropic.
Silicon	20	3.5	0.000	HF HNO ₃	3" Dia.	Si; Steel grey, cubic crystal. High melting point.
Potassium Chloride	21	1.49	34.7 200°C	Alkali Ether Glycerine	7.5" Dia.	KCl; Colorless, cubic crystal. Hygroscopic. Cleaves.
Potassium Iodide	31	1.67	127.5 00°C	Alcohol Ammonia	7.5" Dia.	KI; Colorless, cubic crystal. Very hygro- scopic. Very soft. Cleaves.
KRS-6	23	2.35	0.32 00°C	Slightly soluble in HCl	7.5" Dia.	KRS-6; Colorless, cubic crystal. Toxic.
Cesium Bromide	40	1.70	124.3	Alcohol	1 3/4" Dia.	CsBr; Colorless, cubic crystal. Hygroscopic. Soft. Easily scratched.
Glass	3	1.5-1.6	0.000	HF	No limit	Homogeneous. Colorless. Easily cut, ground, polished. non-toxic.

using potassium bromide, whereby a solid can be mixed with KBr and compressed into a very thin sheet. Since the KBr is transparent in the IR, these discs permit the examination of the solid sample. Preparation of the KBr discs is difficult but opens the way to the examination of a wider range of organic and inorganic materials.

C Gas Cells:

Because of the high sensitivity of the IR Spectrophotometer, it is possible to obtain the spectrum of gases as well as liquids and solids. Obviously the molecular density of gases necessitates a much longer light path. Special gas cells have been developed with path lengths from 5 cm to 10 meters. The 5 cm cells have a single pass light path, while the longer paths are obtained by multiple passes.

IV OPERATING VARIABLES

With modern infrared spectrometers, the operator can control a variety of different recording parameters, the proper adjustment of which depends on the type of spectrum desired. Basically, the performance and accuracy of all infrared spectrometers is limited by the following parameters:

A Resolution

B Accuracy

C Scanning Speed

Control of the above parameters is made available to the operator so that he can choose a set of conditions to enhance his analysis. However, they are not independent, and an improvement in one will be accompanied by a loss in either or both of the other parameters.

The relation existing between resolution, accuracy and scanning speed is of a complex nature and can be expressed quantitatively by the following relation:

$$A^2 S^4 T = \alpha \quad (\text{Luft, 1947}) \quad \text{Equation (-1)}$$

where A is the measuring accuracy,
S, the spectral slit width,
T, the scanning time,
 α , a constant

The spectral slit width represents the approximate small wavenumber interval ($\Delta\nu$) or wavelength interval ($\Delta\lambda$) that passes through the exit slit and is received by the detector. At maximum resolution it is the spectral distance between two bands that can just be resolved by the monochromator (Rayleigh's criterion).

The scan time of a definite wavenumber⁻¹ interval is started in seconds as sec/cm⁻¹ (in the case of wavelength, as sec/ μ).

Now the value of the constant, α , depends on the type of instrument and the frequency or wavelength being scanned. Grating instruments will have a smaller, more favorable constant than prism instruments. This is because of the superior dispersion of a grating instrument. Further, the spectral slit width for a grating instrument remains quite constant over a large wavenumber range - much more so than for a prism instrument.

The measuring accuracy is the ability to read the ordinate scale or sample transmittance (band depth). This accuracy is also affected by the noise level, which is started conveniently in percent transmittance or percent of full scale. A noise of 1% (signal-to-noise ratio - 100:1) is considered adequate for most analysis. It is strictly of a random nature (thermal) and has been shown to originate in the detector. It can be effectively controlled under certain conditions, but it is not considered a primary variable. It is discussed in further detail later.

To summarize, then, there are three variables to consider whenever one plans to obtain the infrared absorption spectrum of a sample: the resolution required, the noise level which is acceptable, and the recording time. The operating conditions used must always represent a compromise between these variables.

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PESTICIDE RESIDUE ANALYSIS OF FISH
BY INFRARED SPECTROPHOTOMETRY

I EXPERIENCE WITH FISH KILLS HAS SHOWN THAT NOTHING LESS IS SATISFACTORY THAN ANALYTICAL METHODS THAT PROVIDE INDISPUTABLE IDENTIFICATION OF THE RESPONSIBLE PESTICIDE.

A Of all the analytical methods for pesticides, infrared spectrometry provides the most positive identification in appropriate applications such as with the chlorinated insecticides.

B There are special problems involved in trying to apply infrared to insecticides of poisoned fish.

1 The interferences are greater than in water; the proportion of total or extractable organic matter to pesticide is high in tissues.

2 Infrared ordinarily requires much greater weights of pesticide than do other analytical methods - 1 or 2 more milligrams.

II TO APPLY INFRARED SPECTROPHOTOMETRY TO FISH TISSUE, NEW TECHNIQUES HAD TO BE DEVELOPED OR EXISTING ONES IMPROVED MARKEDLY.

A Extraction from tissues: The method used must be efficient to give a quantitative estimate. Even more, it must not be selective, so that the analyst can be confident that his results refer to the true composition of the tissues.

B Cleanup of extracts: Infrared demands more purification than do the chromatographic methods, because the latter - such as gas chromatography - include elements of specificity. Even contaminants that do not have interfering infrared spectra must be removed because they interfere with the physical operations of preparing a specimen.

C Micro-infrared techniques: A method that normally requires a milligram of sample was miniaturized to the point that it performs well with 10 micrograms and can get by with 1 microgram. The elements of this technique are:

1 The sample is contained in a micro KBr disc, using great dexterity.

2 The optical path is modified to condense the radiation beam to the effective area of the sample specimen.

3 The spectrum is amplified by electronic scale expansion.

III THE STEPS INVOLVED IN THIS TECHNIQUE ARE:

A Cutting frozen tissue in one-quarter inch squares.

B Macerating tissue in Waring Blendor with hexane.

C Filtering supernatant.

D Concentration of filtered extract on hot water bath.

E Acetonitrile partition of concentrated hexane extract.

F Electron capture gas chromatography after acetonitrile partition.

G Alumina adsorption column cleanup.

H Collection of 50 ml. fractions from alumina column.

I Electron capture gas chromatography of fractions.

J Thin-layer chromatography of fractions.

- 1 Development
- 2 Spraying with rhodamine B dye solution
- 3 Pick up of spots
- 4 Elution of spots with 4:1 hexane/ethyl ether

K Electron capture gas chromatography of T. L. C. spots.

L Infrared Analysis

- 1 Preparation of KBr in porcelain micro boat and putting sample in the KBr
- 2 Pressing micro pellet in 1 1/2 mm disc
- 3 Running spectra - standard vs. sample

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This outline was prepared by Aaron A. Rosen, Chief, Water Sciences Branch, Div. of Field Investigations, EPA, 5555 Ridge Ave., Cincinnati, OH 45213.

PESTICIDE RESIDUE ANALYSIS IN COMPLEX SUBSTRATES
BY INFRARED SPECTROPHOTOMETRY

- I ANALYTICAL METHODS THAT PROVIDE INDISPUTABLE IDENTIFICATION OF THE RESPONSIBLE PESTICIDE ARE A NECESSITY IN WORK INVOLVING POLLUTION.
 - A Despite the use of analytical techniques such as nuclear magnetic resonance, mass spectrometry, gas-liquid chromatography (GLC), and thin layer chromatography (TLC), the unequivocal identification of trace quantities of organic compounds is still best handled by infrared spectroscopy.
 - B There are special problems involved when using infrared identification for insecticides in complex materials, such as fish or animal tissues, sludge or soils containing large amounts of oil or other organics, aqueous industrial effluents, and sewage.
 - 1 There are many interferences; the proportion of extractable organic material to pesticide residue is usually high.
 - 2 For infrared identification in micro-quantities, the separation and clean-up must be very efficient.
 - II WHILE EACH TYPE OF SUBSTRATE MAY PRESENT A NEW PROBLEM, THERE ARE CERTAIN BASIC TECHNIQUES WHICH USUALLY APPLY IN ALL CASES.
 - A Extraction of residue: The extraction should be efficient to give a quantitative estimate, however, there are other instruments which are superior to the infrared for quantitative analysis.

If there are some clues as to the residue in question, the selection of an extraction procedure will be easier.

 - 1 Blending with an organic solvent: This is the usual procedure for tissues. Solvents will vary from hexane to acetonitrile to mixed ethers.
 - B Cleanup and isolation of extracts: Infrared demands more purification than do the chromatographic methods, because the latter - such as gas chromatography - include elements of specificity. Even contaminants that do not have interfering infrared spectra must be removed because they interfere with the physical operations of preparing a specimen.

The combination of column and thin-layer chromatography provides an effective technique for the clean-up and isolation of "trace" amounts of chlorinated hydrocarbon insecticides in complex substrates.
 - C Micro-infrared techniques: Advances in infrared instrumentation, such as beam intensification, scale expansion, better sources and micro-sampling equipment make it possible to obtain satisfactory spectra of as little as 1 microgram of many organics. 50-75 micrograms will give an excellent spectrum without the use of electronic scale expansion.
- 2 Small amounts of tissue; i.e., brains, livers, etc., may be extracted in a micro-blender such as the "Virtis 45"* or macerated with a solvent in small mortars.
 - 3 Oily sludges may be diluted with petroleum ether, the solid material removed, and the liquid portion partitioned with acetonitrile. This step may have to be repeated if large amounts of oil are present. The solids may be extracted in a soxhlet apparatus.
 - 4 Sand and soil samples may be extracted in a soxhlet apparatus.
 - 5 The ultrasonic probe has been successful in extracting organics from clays, this technique has possibilities for many types of samples.

*The Virtis Company, Gardiner, New York
CH, MET, 31, 1, 68

- 1 Samples may be scanned as a solid in a micro-KBr disc, in solution using a micro-liquid cell, or as a solid using a micro-mull plate.

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MMS. January 1963.

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BIOMONITORING OF INDUSTRIAL EFFLUENTS

I INTRODUCTION

- A Plant operating personnel need to know the general quality of an effluent which is being discharged at a fairly constant rate and also must be warned if a slug of toxic material is released to the receiving water.
- B Conventional bioassay procedures can evaluate only single samples taken at particular times. Continuous-flow bioassays of single grab samples over a long period of time can be very useful, but do not solve the problem of transient variations.
- C A technique that does permit exercising continuous surveillance over the toxicity of an effluent is biomonitoring.
- D Conventional bioassays (2, 3, 4) can provide important information about the actual toxicity of batches of the effluent in terms of TL_m's, and, if sufficient samples are tested, about the range of variation. Bioassays should be run from time to time to ascertain the exact toxicity of a waste even though it is being monitored as outlined below.
- E The following procedures refer only to toxic wastes having a relatively rapid action. Wastes such as cadmium which have long delayed, cumulative effects at low concentrations, (5) oxygen-demanding wastes, radioactive wastes, and others, would either be inappropriate or would not elicit a recognizable reaction soon enough to be of use.

II OBJECTIVES OF BIOMONITORING

Three basic objectives of biomonitoring are:

- A To demonstrate the continuous suitability of an effluent for aquatic life provided slow-acting or cumulative toxins are not involved. The continuous testing of an undiluted effluent, (Objective A) is usually accomplished by leading a small stream of the effluent through an aquarium. This aquarium may be located in a public lobby to enhance public relations, or it may be in the plant for operational use only. This is a relatively simple and direct approach and needs no further elaboration.
- B To detect change (usually deleterious) in the biological acceptability of the effluent itself.
- C To detect change in the effect of the effluent on the biota of the receiving water.

III EQUIPMENT

A single basic design of exposure tanks and flow plan can be used to accomplish Objective B or C. (Figure 1) With the exception of a simple suggestion for proportioning flow of effluent to dilution water (Figure 2), engineering devices for accomplishing the various (6, 7, 8) needs outlined below are not discussed. Special care should be used to ensure that all surfaces that come in contact with the waste or the dilution water are constructed of non-toxic and noncorrosive materials. This precaution is particularly necessary for marine waters, where bimetallic contacts are very dangerous. An experienced aquatic biologist should be consulted in the preparation of plans.

A Exposure Tanks

- 1 Exposure tanks (Figure 1) should be large enough (10 to 20 gallons) that the test organisms can live normally under plant conditions. (3) Simple construction

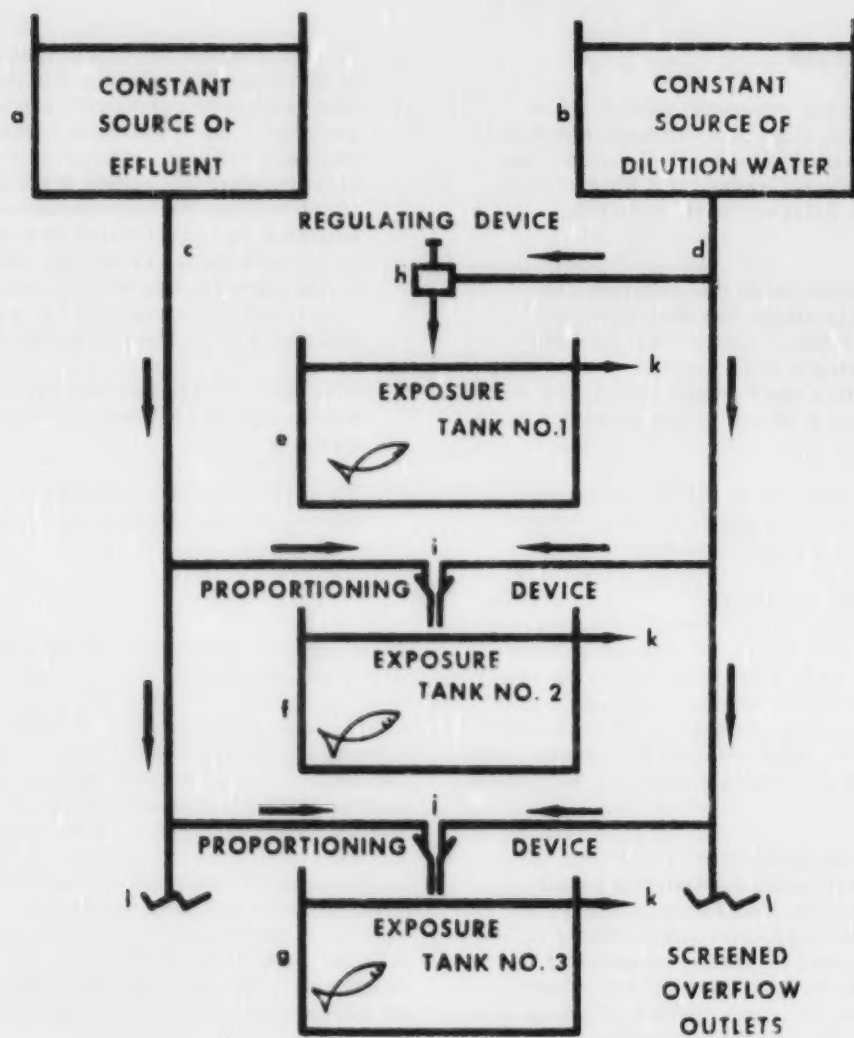


Figure 1. SCHEMATIC FLOW PLAN FOR OBJECTIVES B OR C

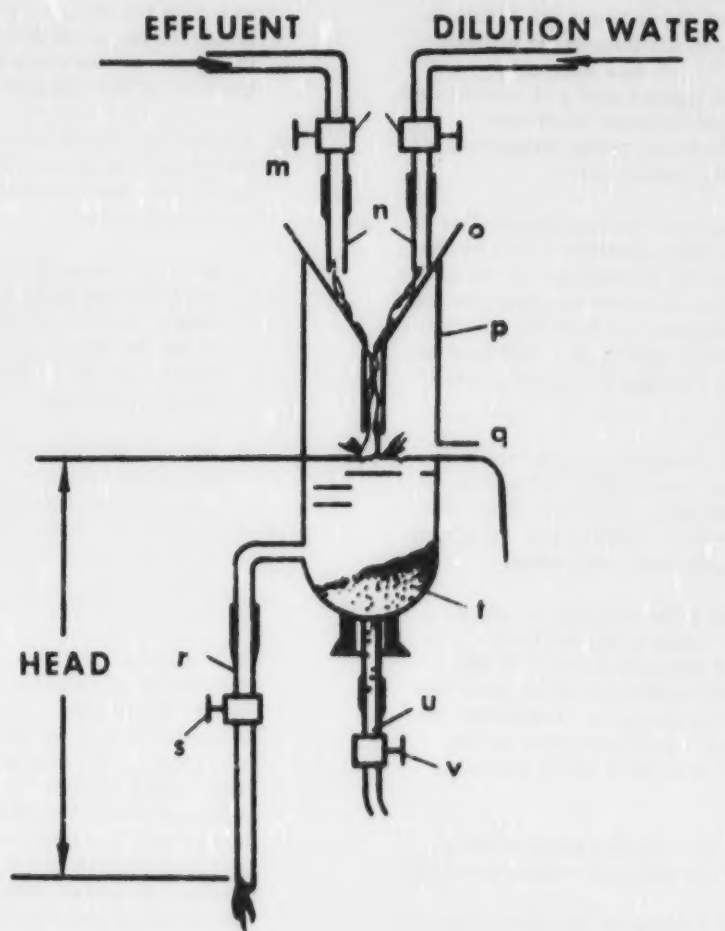


Figure 2

SUGGESTED PLAN FOR PROPORTIONING FLOW BETWEEN EFFLUENT AND DILUTION WATER. (See i and j, Figure 1)

will facilitate feeding, cleaning, and disease control. "Eye-appeal" is not necessary unless public relation is involved, but scrupulous sanitation is essential as in all long-term animal culture. (9, 10) Tanks should be situated in a lighted and well-ventilated room, but not exposed to direct sunlight. Ambient room temperatures are generally satisfactory.

- 2 Inlet and overflow should be similar in all tanks so that conditions will be the same except for the quality of the water. The total flow of water through the tank should be adjusted so that the hydraulic retention times are equal, whether the total flow is coming from one source or two.
- 3 Tank No. 1 contains unadulterated dilution water to establish that the test animals will live in it. This tank is the control or "reference" to which the other tanks are compared.
- 4 Tanks 2 and 3 (more may be added at points 1) contain mixtures of effluent and dilution water. If the experimental animals die or show distress in these tanks, a change for the worse in the characteristics of the effluent being monitored is probably indicated.
- 5 i and j are mixing or proportioning devices set to predetermined amounts.
- 6 All tanks overflow to the sewer through opening k, which should be screened to prevent escape of test animals and clogging in outlet pipe.

B Effluent Supply

The supply of effluent should be constant and controllable. The prime requisite is that it be fresh so that changes may be detected at the earliest possible moment.

C Dilution Water

- 1 The source and quality of the dilution water is the most important single factor in the system because this is the scale or standard by which the toxicity of the effluent is assayed.
- 2 To test the effect of the effluent on the receiving water (Objective C), the dilution water obviously must be the receiving water.
 - a In most cases the receiving water varies from time to time as runoff water washes in different materials from the surface of the land the tide turns, or other plants release various wastes.
 - b Plan C (Objective C) automatically evaluates the toxicity of the effluent when discharged into this changing situation. This water should contain all of the components present down to the outfall being monitored, but none of the effluent itself.
- 3 In a stream situation, dilution water can be taken well upstream from the waste discharge. A pipeline with a continuous flow is ideal, since slugs of material from upstream sources that might modify the toxicity of the effluent being monitored would be taken into the monitoring system and quickly distributed to the exposure tanks. Because such systems are notoriously subject to "problems," batch transportation of control water may have to be employed. In lakes, estuarine, or coastal situations, batch supply may be the only practical solution.
- 4 If the receiving water is already continuously toxic to aquatic life, it is unsuitable for use in the system.
 - a Under such circumstances Plan C would be unworkable and the only recourse would be Plan A or B.

- b Treated water such as drinking water from a city or industrial plant should never be used for any of these plans, even if dechlorinated, because the chemicals used in treatment may react with the waste being monitored.

D Proportioning Flow

The plan (Figure 2) by which effluent and dilution water are proportioned to the test tanks (Tank 2 and 3) determines what the system will accomplish.

- 1 A suggested plan for proportioning flow between effluent and dilution water (i and j) in Figure 1 is given in Figure 2.
- 2 Rubber tubes (n) can be momentarily diverted to catch flow in an appropriate sampling device such as a graduated cylinder. Based on the time required to discharge some standard volume, flow could be proportioned to any desired ratio (for example, one part effluent to two parts dilution water).
- 3 The removal of sediment by some sort of trap as shown is a policy matter that should be resolved in the project-design statement.

IV FLOW PLANS

A Flow Plan for Objective B

If the system is to be operated to detect only serious detrimental change in the effluent itself, Tanks 2 and 3 should contain mixtures in such proportions of effluent and dilution water as to permit the test animals to live as long as the effluent is normal.

- 1 One possible combination would be to adjust the mixing mechanism at i (Figure 1) to admit such a proportion of effluent that the test animals in Tank No. 1 could barely survive. The slightest increase in toxicity of the

effluent would then immediately be made evident by the death of the test animals and appropriate remedial action could be taken.

- 2 The mixing mechanism j in Tank No. 3 might be adjusted to provide a greater margin of safety, for example 1/2 or 1/10th the toxicity of Tank 2. If Tank 2 animals then died, but Tank 3 animals survived, it would presumably indicate only a moderate increase in toxicity.

B Flow Plan for Objective C

Monitoring the effect of the effluent on the receiving water, from the point of view of protecting aquatic life, is ideal, but may also be very difficult.

- 1 The reference tank (e, Figure 1) receives water fresh from the receiving body. This water is free of any trace of the effluent being monitored, but contains all substances, natural and artificial, presently in the receiving water. These components may change from time to time, and one of these changes may increase the toxic effect of the effluent (synergism).

Thus the death of animals in the strongest test tank, but not in the reference tank, may be the result of an increase in the toxicity of the plant waste, or of a synergistic reaction of the effluent with a material in the receiving water.

- 2 No matter the cause, the death itself serves as a warning and immediate action can be taken on the discharge to protect aquatic life.
- 3 On the other hand, if a slug of strongly toxic matter enters the receiving water from some outside source, the deaths of the animals in the reference Tank 1 (and probably also those in exposure Tanks 2 and 3) would show that the "fish kill" presumably in progress in nature was not the result of the effluent.

- 4 A parallel installation under Plan B might also demonstrate no change in the waste being monitored.
 - 5 Numerous other dilution systems might also be employed under Plan C.
- C A different approach is to proportion the mixture to simulate the actual mixture taking place in the receiving water. For this purpose if the location were on a flowing stream, the flow of both the stream and final effluent must be known. Periodic adjustments might be made by hand, or by automatic equipment involving telemetry of both effluent and stream flow.

V TEST ANIMALS AND OTHER CONSIDERATIONS

No universal recommendations can be made about test animals to be used although many suggestions are available. (1, 3, 5)

A Irwin⁽¹¹⁾ investigated the suitability of 57 species of freshwater fishes for this purpose. Briefly, they should be of local importance, they must be a type that can be maintained in good health in the laboratory in the dilution water to be used, and enough must be employed so that reasonable statistical reliability is assured (for example, 10 per tank).

- 1 Fish are usually employed as test animals, although there is no reason not to use any other organisms that can be successfully kept alive in the test tanks. (12)
- 2 Continuous availability is also important. It is well not to change the test species after a program has been established, since the reactions of different species to the waste being monitored may not be the same. (11)
- 3 Animals in the exposure tanks should be fed the same as those in the stock tanks. The same kind of food in the same ratio of food to weight of test

fish should be added to each tank at the same intervals. Unnatural acceptance of food in exposure tank may indicate a measure of distress, even in the absence of mortality.

B Oxygen determinations should be run occasionally to ensure that any deleterious symptoms are the result of the effluent and not of oxygen deficiency. Actual minimum acceptable levels will depend on the temperature and type of fish used.

C Long-continued exposure to low level concentrations in tanks may result in cumulative intoxication, or acclimatization.

- 1 In most cases these effects can probably be best counteracted by periodic renewal of the test fishes, for example: at 60-day intervals.
- 2 When obtaining stocks of test animals from receiving waters, these same factors should be borne in mind. Fish or other organisms taken from below the outfall might have acquired some immunity or sensitivity to the effluent being tested.
- 3 Fish or a species normally present in the receiving stream, but imported from some other (unpolluted) source, will presumably exhibit a completely unconditioned response. Generally, since the aquatic life to be protected is that already present in the stream, the most logical source of fish is the stream itself. Under operating conditions, however, it is not always practicable to collect the experimental animals from this source and imports from another area may be necessary.

D Selection of Dilutions

- 1 The "critical range" of toxicity may be defined as the range between the highest concentration that kills no test animals and the lowest concentration that kills all. The TL_m of the conventional bioassay⁽³⁾ is in the middle portion of this range.

2 When an effluent is to be biomonitoring under Plan B, the selection of appropriate dilutions might be based on the above concept of "critical range."

a If a "tight" control is desired, the highest concentration might be established near the TL_m . When a batch of test animals is first placed in such a dilution, approximately half of them may (by design) be expected to die. The survivors, however, would constitute a rigorous control as any increase in toxicity would be expected to kill the remaining animals in order of susceptibility, until, as the top of the critical range is reached, all would be dead.

b A somewhat less stringent control would be effected with a dilution near the lower end of the critical range.

3 In any large population of test animals kept in an exposure tank over an extended period of time, an occasional animal may be expected to die.

a The mortality of significance then is not the occasional individual death, but the sudden death of 25, 50, or 100% of the test animals.

b When this happens, biomonitoring has sounded the alarm to take appropriate action to detoxify the effluent or to divert it from the receiving stream until it is again normal.

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METHODS OF DETECTION OF CHOLINESTERASE INHIBITION

The first step is to see what cholinesterase is and why it is important. Cholinesterase (ChE) is unique because it is known to be the target or site of action of the organophosphate and carbamate pesticides. Therefore, we can measure the effect of these pesticides at their site of action, which can't be done with chlorinated hydrocarbons because their exact target hasn't been pinpointed yet.

The biological importance of cholinesterase is that it functions in the nervous system, which is, of course, extremely important to higher organisms. Some poisons, whose primary target is elsewhere, commonly produce their ultimate effect upon the nervous system: Heart poisons, or poisons which affect the oxygen-carrying capacity of the blood are lethal because of brain damage which results from the brain's great oxygen requirement not being met.

Many other poisons act directly on specific parts of the nervous system, and the reason they cause problems is that the nervous system can't tolerate even brief interruption. Compounds which act only briefly on other tissues may have little effect because of the ability of other tissue to withstand brief periods of interruption without affecting the function of the organism as a whole. In virtually every case where we understand the basis of insecticidal action, the effects are directly upon nerve.

Cholinesterase is important because it breaks down acetylcholine (ACh), and acetylcholine functions in the nervous system. How ChE breaks down ACh will be discussed later. First, what does ACh do.

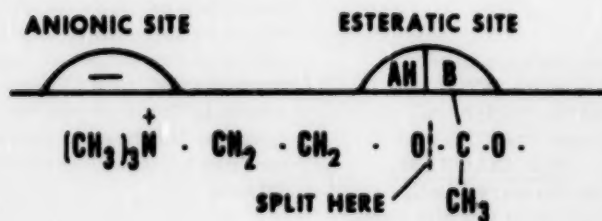
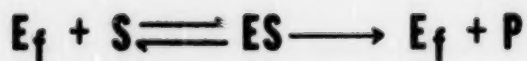
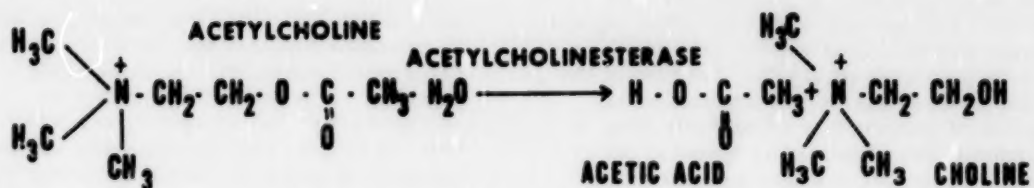
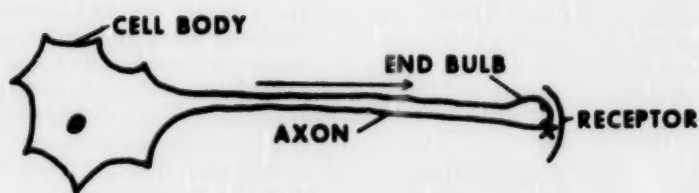
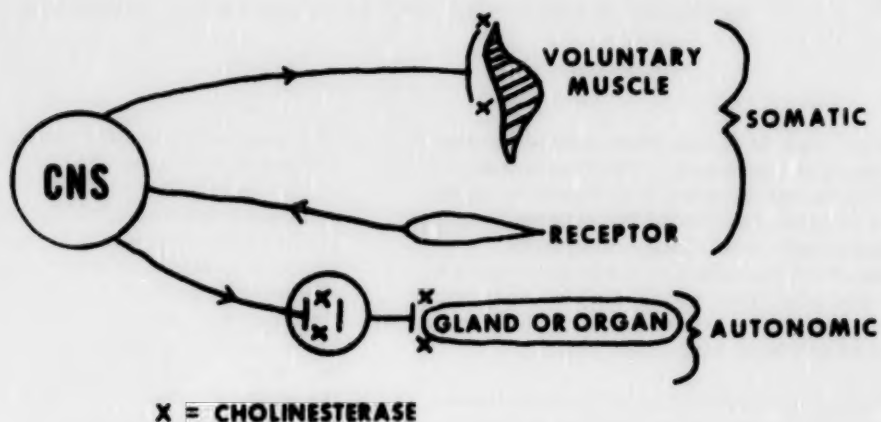
The nervous system in vertebrates is made up of the central nervous system (CNS) or brain and spinal cord, and the peripheral system or the nerves between the CNS and the muscles, glands, etc. SEE DIAGRAM. The basic unit of nerves is the nerve cell, which is made up of the cell body and the axon. SEE DIAGRAM.

The long nerves which can actually be seen are bundles of axons.

Nervous impulses travel along nerves electrically, but when they get to a junction which is called a synapse, they can't cross electrically and what is called synaptic transmission occurs. This process is chemical and is where acetylcholine comes in. When a nerve impulse, traveling along the axon, reaches a synapse, the impulse dies out. But, it causes the release from the end of the axon, of a small amount of a chemical, the transmitter substance, which crosses the synapse and triggers either: 1) another impulse if the synapse is between nerve cells, or, 2) a response if there is a receptor there such as muscle. There are two known transmitter substances; 1) acetylcholine at cholinergic synapses and 2) noradrenaline at adrenergic synapses. Acetylcholine is the major one, with noradrenaline being found in only a small part of the system.

After a transmission, the transmitter must be eliminated so that the receptor can return to a resting condition. At cholinergic junctions, it is promptly removed by cholinesterase, which hydrolyzes it to inactive acetate and choline. The cholinesterase is stored in clefts on the end bud.

When ACh is destroyed, it must be done rapidly, and this is accomplished by an enzymatic reaction. Acetylcholinesterase is an enzyme and by enzyme nomenclature, it generally tells what it does. AChE means an enzyme which acts on ACh. There are two types of ChE: 1) AChE or true ChE which is the one we are interested in and which is the target of organophosphates and carbamates, and 2) pseudocholinesterase which is found primarily in vertebrate blood plasma, but has no known biological function, and can be totally inhibited without harmful effects.



An enzyme is a biological catalyst, that is, it changes the rate of a particular reaction or group of reactions, without being changed itself. The reaction involved here is the splitting of ACh into choline and acetate. SEE DIAGRAMS. The bond which is split is called an ester linkage and so, the enzyme is called an esterase. The reason that an enzyme is so specific is that it has certain active sites on it which in natural situations are found only on the compound on which it acts. SEE DIAGRAM. After the substrate molecule is split, the original conditions of the active sites are restored and the enzyme is ready to be active again.

What we are really concerned with is the action of organophosphates on the enzyme. There are two classes of inhibition of an enzyme: 1) Competitive or reversible, and 2) non-competitive or irreversible. The classification is based partly on the regeneration rate of the inhibited enzyme. Competitive inhibitors are usually structurally similar to the normal substrate. The inhibitor combines with the active site, but does not continue into a further reaction, and when the inhibitor is removed the enzyme returns to normal activity in a short time.

AChE inhibited by a carbamate returns to normal a few hours after removal of the excess carbamate; AChE inhibited by an organic phosphate, however, returns to normal only after several days. The difference is that the inhibition by an organic phosphate is not due to competitive adsorption on the enzyme surface, but is a result of an irreversible chemical reaction in which the enzyme is phosphorylated. The enzyme can no longer break down all of the ACh at the synapses and the organism is in trouble.

The symptoms of organophosphate poisoning are what one would expect if ACh is allowed to remain at the synapse. Initially there is excessive activity at the synapse, but then there is a block. Usually there is contraction of the pupils, slowing of the heart, dropping blood pressure and other symptoms. If poisoning is severe, convulsions occur. Death is due to respiratory failure and lack of oxygen.

These last comments have been reported for vertebrates. In insects, much less is known. Excitability does occur, and paralysis and death, but, respiration is mainly by passive diffusion and insects are very resistant to lack of oxygen, so the immediate cause of death is completely unknown.

DETERMINATION OF CHOLINESTERASE

Cholinesterase inhibition is still in effect when the tissue containing the enzyme is removed from the intact animal. Therefore, we can just measure the activity of the enzyme in organisms suspected of being poisoned and compare it to the enzyme from a known normal organism of the same species. When the sacrifice of the whole animal is not practical, such as with people, the activity of the enzyme in the red blood cells can be used. It is the same as that found in the nervous system. In your work today, you will use fish brains. In most fish the brain can be removed whole very easily and has high AChE activity.

There are many methods of measuring AChE inhibition. The main ones are:

- 1 pH change method
- 2 Titrimetric method
- 3 Measurement of unchanged acetylcholine
- 4 Radiometric method

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PRINCIPLES OF COLUMN SELECTION AND PREPARATION

Part 1

I INTRODUCTION

The purpose of this discussion is to introduce basic information about supports, phases, and column packings. This knowledge can prove to be an adjunct to better gas chromatograph operations, and clearer thinking about separation problems. No complex mathematical theory will be presented. An attempt is made, however, to present the ideas of liquid phase selectivity using the relative retention concept and elution order. Common organic compounds will be used as examples. This is followed by a treatment of some pesticides. Physical methods of packing preparation, column packing, and column conditioning will be described with special emphasis on our company methods.

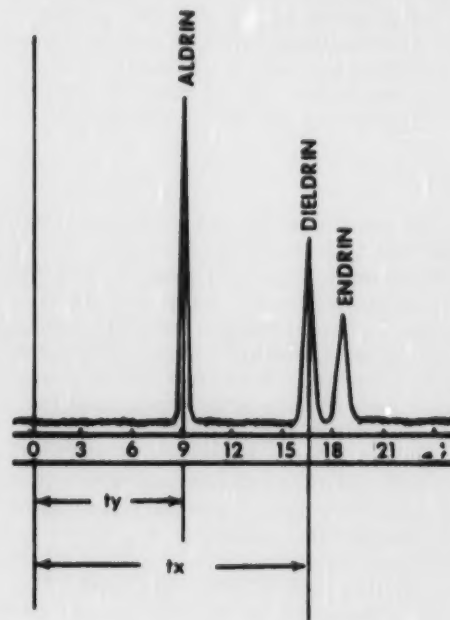
II DEFINITIONS

- A Solid Support - A solid, or correctly, a porous solid of some form which acts as a retainer for the stationary phase.
- B Stationary Phase - A liquid or low melting solid, usually an organic or silicone compound, which is in the liquid state at operating temperatures.
- C Mobile Phase - An inert gas which acts as the moving phase.
- D Gas Liquid Chromatography - Mixture separations are achieved by interaction of the components between the liquid phase and the mobile gas phase. The general term Gas Chromatography or GC will be used throughout this discussion.
- E Solid Stationary Phase - Solid phases at operating temperature which are self supporting.
- F Gas Solid Chromatography - Mixture separations are achieved by interaction

of the components between the surface of the solid stationary phase and the mobile phase.

The subject of Gas Solid Chromatography will not be discussed, since it has only limited application to the pesticide analyzer.

- G Pesticide Analyzer - Anyone who analyzes samples for pesticides.
- H Relative Retention Time - A numerical expression of the elution order to two components.



Relative Retention Time or $tr = \frac{tx}{ty}$ where tx and ty may be expressed in any convenient consistent units, i.e., seconds, minutes, inches of chart paper, cm of chart paper, or if you desire, "pico furlongs per fortnight".

NOTE: That t_x and t_y are measured from the solvent peak and not the point of injection. There is a mathematical justification for this, but in keeping with my promise, we will not discuss it. See Dal Nogare & Juvet, Gas Liquid Chromatography, Interscience, New York, 1962.

III SOLID SUPPORTS

A Glass Beads

There are three types available. Regular glass beads, etched surface beads, and porous glass beads. The surfaces can be deactivated. High pressure drops, low phase loading, and small sample capacity are characteristics of glass bead type supports. Except for porous glass beads, these materials offer low surface area and do not produce highly efficient columns. The porous glass beads, however, show great promise for future use in GC. Glass beads are not a support of choice for most pesticide applications.

B Teflons

There are three popular supports within this category; Screened Teflon 6 (Chromosorb T), Fluoropak 80, and Kel-F. Low pressure drops, moderate phase loading, normal sample capacity, and surface inertness are characteristics of these supports. Screened Teflon 6 and Fluoropak 80 have wide application for the analysis of highly polar compounds. Maximum column efficiency of 350 plates per foot can be achieved only with careful handling techniques, proper phase loading, and low gas flow rates, (i.e., 15-30 cc/min from gas pressures of 1-5 psig at the inlet). For further details see: Kirkland, J.J., *Anal. Chem.* **35**, 2003, (1963); *GAS CHROM Newsletter*, Appl. Sci. Labs., Inc., Vol. 8, No. 3, July, 1967.

C Diatomites

- 1 Calcined Diatomite - Firebrick - Red
Compressed Diatomaceous Earth in brick form is fired above 900°C and

turns red during calcination. The bricks are crushed and ground, then screened into mesh cuts.

- a Advantages - Great surface area, high efficiency columns, particles are moderately strong.
- b Disadvantages - Extremely active surface - does produce decomposition of sensitive samples, isomerization, reversible and irreversible adsorption. Not recommended for many pesticides such as Heptachlor, DDT's, Endrin, Trithion, Malathion, Imidan, Imidoxon, and Dyfonate. It is difficult to remove dust from this support; cannot be well deactivated.

2 Flux Calcined Diatomite - Celatom & Celite - White

An intimate powdered mixture of Diatomaceous Earth and sodium carbonate is calcined around 900°C. The product is air classified and screened to produce final product mesh cuts. Second most efficient columns are produced by this type of support.

For an excellent discussion about support physical characteristics, see: Ottenstein, D.M., *J. of Gas Chromatog.*, **1** (4), 11, (1963).

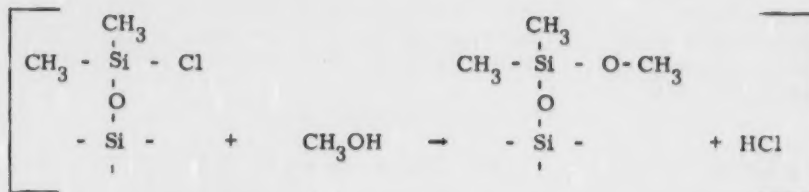
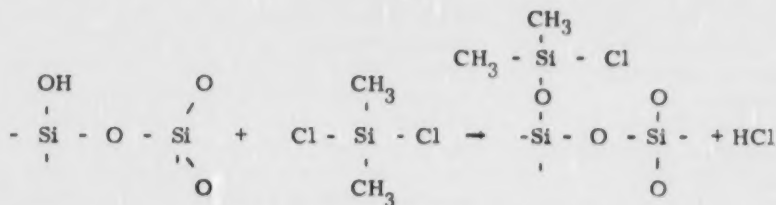
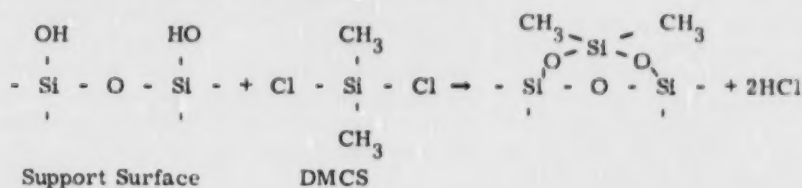
- a Advantages - Can be treated to make the surface inert and yet maintain good efficiency.
- b Disadvantages - Moderately active surface, particles are weak.

D Treatment of Diatomites

- 1 Acid Washing - Concentrated HCl is used by all manufacturers which is thought to remove surface iron salt contaminants. Changes pH of white supports from 8 to about 7. Neutralizes K_2O , Na_2O , and NaOH present in white support. Usually 2-5 days is sufficient.

- 2 Base Washing - Removes any organic matter that might be present.
- 3 Silane Treatment - The critical step - this method of deactivation has proven itself by the test of time.

DMCS (Dimethyldichlorosilane) is used exclusively by the support manufacturers for production of top notch deactivated white supports. Supports prepared by silanization with DMCS have worked for such sensitive compounds as steroids, complex drugs, and pesticides. Chemically speaking, there are two reactions theorized. The surface OH groups react with DMCS in this manner:



For details see: Supina, Henly & Kruppa, J. Am. Oil Chem. Soc., 43, (5) 202A, (1966). Applied Science Bulletin #12.

E Solid Stationary Phases

1 Molecular Sieves, Silica Gel, Charcoal, Alumina

Used for gas analysis and low boiling hydrocarbons, and a host of other items. Not satisfactory for most pesticides because of high surface activity.

2 Porous Polymer Beads

These materials are highly cross-linked polymers of styrene and divinyl benzene. The polymers are precipitated in spherical form from solvents. They are thought to be inert, and have a wide range of application for the analysis of highly polar materials. Unfortunately, they are limited to low boiling compounds and low molecular weight materials. For example, the C_6 hydrocarbons can be analyzed on a 6 foot packed, Diatomaceous Earth column below $100^\circ C$; on the Porous Polymer Beads, a temperature of $170^\circ C$ is required. They are not of great interest in pesticide analysis because of this limitation.

SEE: Hollis, O.L., Anal. Chem. 38, 309, (1966); Johns-Manville Bulletins FF-157A, FF-177, and FF-181.

IV THE SUPPORT OF CHOICE FOR PESTICIDE WORK

A white calcined Diatomaceous Earth support which is: acid washed, properly silane treated, quality controlled for pesticide work, reasonably consistent from batch to batch. Our choice is the 100/120 mesh cut of GAS-CHROM Q.

Performance properties are shown in Applied Science GAS-CEROM Newsletters, April - June, 1965, Vol. 6, No. 2-3 and May, 1968, Vol. 9, No. 3.

V STATIONARY PHASES AND SELECTIVITY

Based on the present state of the art, column selection for any separation problem is dependent upon the availability of retention data. When this data is unavailable, most people consider the selection of a proper column to be a hit and miss proposition. This is not the case, because a person with experience can make a highly educated guess which will be right more often than wrong. Much of this experience can be quickly gained by application of the phase selectivity concept.

We will first introduce the fundamentals of elution order and selectivity as applied to simple compounds. We will then apply the general rules of phase selectivity to some simple pesticide separations. The relative retention concept will be used in the second section and tables of retention data supplied for your future use.

Classification - The method of classification of stationary phases is based on separation characteristics. Two broad classes do exist. Work is being done in the U.S. and in Europe based on a systematic approach to classification of phases by selectivity using basic reference compounds. All systems do require retention data of some form or another, both on the stationary phases used and the compounds analyzed. Two systems are evolving which might be of interest to you. Since both methods require somewhat complex mathematical treatment, we will not discuss them. Anyone interested should study Anal. Chem. 36 31A-41A, (1964) and Rohrschneider, J. of Chromatog. 22, (1966). The latter paper is in German, unfortunately.

A Non-Selective Phases

The first group which we should discuss is the non-selective (often named the non-polar) category. All phases within this group usually separate components on the basis of boiling point, or volatility. As a general rule, compound structure

differences cannot be differentiated by the non-selective phases unless those structure differences can result in a distinct difference in boiling point or volatility. Many compounds which decompose at, or below, their boiling point or do not have a measurable boiling point can still be separated by non-selective GC phases. Vapor pressure information would then be the determining factor in elution order. Some typical examples within the small non-selective category are Apiezon L, SE-30, DC-200, and OV-101.

Some typical elution orders are presented below along with the boiling points of the compounds where known.

Alkyl Compounds
Phase SE-30 or OV-1

Elution Order	BP°C
1-Butene	- 5
n-Butane	0
1-Pentene	30
n-Pentane	36
1-Hexane	63
n-Hexane	69

Aryl Compounds
Phase DC-200 or OV-101

Elution Order	BP°C
Benzene	80
Methyl Cyclohexane	100
Toluene	110
Ethyl Benzene	136
n-propyl Benzene	159
n-butyl Benzene	183

Fatty Acid Methyl Esters

APIEZON L

Methyl Linolenate
Methyl Linoleate
Methyl Oleate
Methyl Stearate
Methyl Arachidate

Mixed Compounds
(Analogous to Mixed Pesticides)
APIEZON L

n-Pentane	36
Chloroform	61
Carbon Tetrachloride	77
Ethanol	78
Benzene	80
Methyl Cyclohexane	100
Toluene	110
Chlorobenzene	132
Ethylbenzene	136

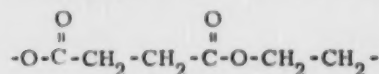
B The Selective Phases

The second and by far the largest group may be called the selective phase group. The phases in this category separate components not only by boiling point but to a great degree by structural differences. It is not unusual for lower boiling compounds to elute after higher boilers from columns prepared from selective phases. We may call this behavior "selectivity". The phases in this group are almost endless since just about anything from pentanol to peanut butter can be used. Both materials do exhibit selectivity, by the way.

Some phases are more selective than others and this can be predicted to some degree with a basic knowledge of organic chemistry. The greater the electron shift or polarity of the phase molecule, the greater will be its "Selectivity"; thus, EGS is more selective for unsaturation than OV-17 than SE-30. A phase which contains groups similar to a material being analyzed will retard the compound. This retardation can be remarkable at times. We might assign the term "Phase Affinity" to describe this behavior although it is really a special form of selectivity. In actual practice most selective phases will exhibit both kinds of behavior.

C Typical Selective Phases of Great Popularity

EGS is a typical polyester phase which exhibits selectivity toward unsaturation. The basic repeating unit of this polymer is highly polar and looks like this:



1, 2, 3-Tris (2 cyanoethoxy) propane exhibits greater selectivity than EGS. The group, $-\text{O}-\text{CH}_2-\text{CH}_2-\text{C}\equiv\text{N}$, on this molecule and the phase's symmetrical structure make it highly selective for unsaturation.

OV-17 is a 50% phenyl substituted silicone (structure shown on page 12). The phenyl groups show a distinct phase affinity toward the benzene ring and some selectivity toward double bonds. It is selective toward other unsaturation but not nearly as much as EGS.

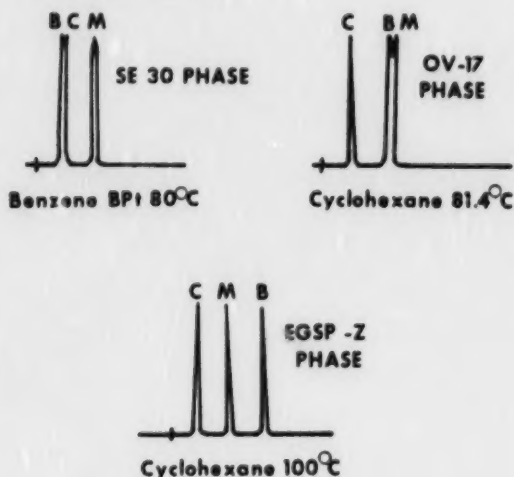
EGSP-Z contains both phenyl silicone and the basic EGS units. It exhibits selectivity for unsaturation almost as great as EGS and phase affinity like the OV-17.

Based on experience, we can list these and other phases in descending order of selectivity: trispropane, EGS, EGSP-Z, OV-17 and SE-30. Typical elution orders are shown on the selective phases described.

ELUTION ORDER EXAMPLES

<u>Selectivity on EGS</u>	<u>Selectivity on 1, 2, 3-Tris (2-cyanoethoxy) propane</u>	<u>Phase Affinity on OV-17</u>
Me Stearate	n-pentane	Cyclohexane
Me Oleate	1-pentene	Benzene
Me Linoleate	n-hexane	± Methylcyclohexane
Me Arachidate	1-hexene	
Me Linolenate	n-heptane	
	1-heptene	
 <u>Selectivity & Phase Affinity on EGSP-Z</u>		
	Cyclohexane	
	Me Cyclohexane	
	Benzene	

A dramatic demonstration of the relative selective characteristics of SE-30 (non-selective), OV-17, and EGSP-Z should serve to illustrate the point. The three drawings below show the elution order of Benzene (B), Cyclohexane (C), and Methylcyclohexane (M) on three different columns. The retardation of benzene by the EGSP-Z phase is equivalent to raising the boiling point of benzene by 40°C. Note that OV-17 retards benzene to the methylcyclohexane position and EGSP-Z retards and elutes benzene well after methylcyclohexane.



The rules, or perhaps better, the guidelines we have illustrated are general and therefore subject to exception. Anyone who desires an example of an exception should examine the behavior of ethylacetate and isopropyl alcohol on Apiezon L and Carbowax 400.

VI PHASE REQUIREMENTS FOR PESTICIDE ANALYSIS WORK

A stationary phase must possess three characteristics to be useful for pesticide analysis studies.

- 1 It must produce little or no bleed at operating temperatures to minimize detector contamination. Most pesticides require analysis temperatures above 180°C.
- 2 It must separate the pesticides that are encountered in the sample.
- 3 It must not act as a catalyst for pesticide decomposition, nor can it be chemically reactive with the pesticides.

These three requirements eliminate the bulk of phases available for use. Except for a few isolated cases, the silicones offer sufficient range of selectivity to solve most pesticide separation problems, and in addition, offer the other desirable characteristics listed above.

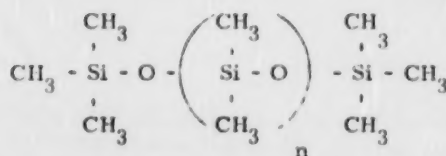
The most commonly employed column for this work is a 6 foot $\times$ 3, or 4mm I.D. glass column packed with 10% DC-200 on GAS-CHROM Q. With the addition of two other columns in the arsenal, most pairs of pesticides can be done by careful column selection. We suggest the use of 10% OV-17 and 10% QF-1 columns for this application. In addition, two screening columns of DC-200/QF-1 and OV-17/QF-1 can also be considered part of the basic arsenal. The article on the next page about silicone phases is supplied for your study. If greater selectivity is required than is available in the five columns listed previously, we suggest the consideration of a 5% OV-225 column. This is the most selective silicone phase available today which meets all the phase requirements for pesticide analysis.

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SILICONE STATIONARY PHASES

Confusion exists among many gas chromatographers as to the structure and separating characteristics of silicone compounds presently being used as phases in gas chromatography. Many users insist that only one specific methyl silicone phase will achieve a certain separation. This may be true in a few rare instances but it is a fallacious general conclusion.

The basic structure of all silicones is actually very simple and for any methyl silicone appears as follows:



The quantity n , which determines the molecular weight of any chain, can range from zero to many thousands. Silicone oils and gums contain mixtures of chains of various molecular weights; the higher the molecular weight of the average chain the higher the viscosity of the silicone oil or gum. The oils and gums can be cross-linked by using an appropriate catalyst, but the resulting compound is a silicone rubber, not normally used in gas chromatography as a liquid phase.

The temperature stability and volatility of a silicone phase in a packing are dependent on three factors: 1) impurities present in the oil or gum; 2) activity of the solid support; 3) molecular length of the chain. Molecular size has no significant effect on the volatility of a high purity silicone if the molecular weights of the chain are such that the vapor pressure is negligible at the operating temperature. Long chain methyl silicones can be produced which have negligible vapor pressures close to their decomposition temperature (which approaches 400°C).

Since it is possible to analyze methyl silicones having molecular weights in the range 2000 to 3000 on short 3% SE-30 columns, it would appear that the molecular weights of methyl silicones having measurable vapor pressures close to 400°C extend to rather high values (i.e. 10,000 or more). Consequently, these shorter chain compounds appear as bleed when methyl silicone columns are used at elevated temperatures in gas chromatography. The methyl silicones most commonly used are the following: DC-200, E301, GE SE-30, GE SF-96, JXR, OV-1, UC L-45.

It must be emphasized that these phases should give equivalent separations for almost all compounds. Any slight variations can be attributed to impurities present in the silicones themselves or to the effects of the solid support.

Methyl groups along the chain can be replaced by other organic substituents. For purposes of this discussion percent substitution will be considered as a percent group replacement. For example, if all methyl groups are replaced by phenyl groups this will be called 100% phenyl substitution.

VINYL SUBSTITUTED SILICONES

These substituted silicones possess the same basic structure and properties as the unsubstituted compounds, the only difference being the presence along the chain of vinyl groups in place of some of the methyl groups. The percent of side chain vinyl groups is quite low (less than 1%) because the vinyl groups can open and crosslink between chains at elevated temperatures.

The following vinyl substituted silicones can be expected to give separations equivalent to those obtained with methyl silicones: GE SE-31, GE SE-33, UC W-96 and UC W-98. SE-31 and SE-33, no longer

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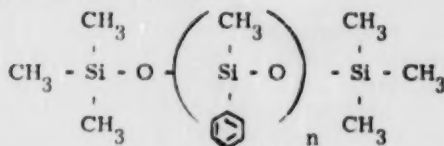
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readily available, will be discontinued by us when the present supply is exhausted.

PHENYL SUBSTITUTED SILICONES

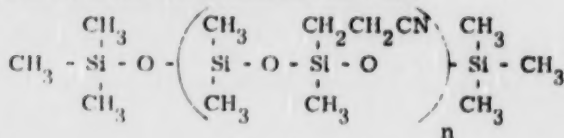
The replacement of some side chain methyl groups on the silicone chain by phenyl groups produces a "polar" or "selective" silicone phase. Since the benzene ring is inherently stable to 400°C., the phenyl substituted silicones should exhibit approximately the same temperature stability as the methyl silicones. The degree of "polarity" or "selectivity" is determined by the percentage of the methyl groups replaced by phenyl. Phases which fall into the above category include: DC-550 (about 25% phenyl), DC-560 (unknown, but thought to be about 5%), DC-710 (about 50% phenyl), GE SE-52 (approx. 5% phenyl), GE SE-54 (approx. 5% phenyl plus less than 1% vinyl), GE Versilube F-50 (about 10% tetrachlorophenyl), OV-17 (50% phenyl).

The basic structure of a 50% phenyl-substituted methyl silicone (such as OV-17) appears as follows:



NITRILE SUBSTITUTED SILICONES

An even more selective silicone phase is produced when cyanoethyl groups ($-\text{CH}_2\text{CH}_2\text{CN}$) are substituted for methyl on the silicone chain. Unfortunately, the cyanoethyl methyl silicone has a far lower decomposition temperature than the methyl silicone because of the nitrile group. Nitrile substituted phases include GE XF-1105 (5% cyanoethyl), GE XE-60 (25% cyanoethyl), and GE XF-1150 (50% cyanoethyl). From this discussion, XE-60 would be structured like this:



OTHER SUBSTITUTED SILICONES

"Polarity" or "selectivity" can be achieved by replacing methyl groups with other side chain groups besides phenyl and nitrile. Only two silicone phases which have shown wide popularity fall into this category. They are DC QF-1 (probably 50% trifluoropropyl) and DC LSX-3-0295 (probably 50% trifluoropropyl plus less than 1% vinyl). From the limited work we have done, it is apparent that there is no difference in the cholesterol/3 β -cholestanol separation when run on 3% packings prepared from either of these two phases. A higher temperature limit has been suggested for LSX than for QF-1; this is more likely due to product purity rather than the presence of the vinyl side chains.

CHAIN ALTERATION

Another method of changing the structure of silicones is to introduce polyesters into the backbone chain of the silicone (or introduce silicone groups into the polyester chain). Here, however, the polyester component dictates the stability of the resulting silicone or organosilicone phase. These phases exhibit the greatest selectivity of all the above mentioned compounds but also possess the lowest usable temperatures. The polyester portion of the chain in the following compounds is ethylene glycol succinate:

ECNSS-M (approx. 30% cyanoethylmethyl silicone in the chain), ECNSS-S (approx. 5% cyanoethylmethyl silicone in the chain), EGSP-A (approx. 5% phenyl silicone in the polymer chain), EGSP-Z (approx. 30% phenyl silicone in the chain), EGSS-X (approx. 5% methyl silicone in the chain), EGSS-Y (approx. 30% methyl silicone in the chain).

It is readily apparent that a wide variety of selectivities, or polarities can be realized from this fine collection of liquid silicone phases, all of which we sell. It is also evident, however, that in the vast majority of cases several phases of the same type will give equivalent separations.

PART 2

In the November, 1967, GAS-CHROM Newsletter we discussed at some length the structural differences between many silicone stationary phases. During the past year new and exciting silicones have been developed which increase the number of quality phases for use in gas chromatography.

The most notable developments are OV-101, General Electric GC grade SE-30, OV-210, OV-225, and OV-25. These silicone phases were all designed for maximum stability at the highest possible temperature. We want to discuss the structure of these new phases and, in addition, pass on some new and vital information about silicone phases in general.

PHASE STABILITY

Inorganic bases are the worst enemy of silicone stability at elevated temperatures. Even 0.1% base will cause rapid and complete degradation, or "unzipping", of a silicone molecule at temperatures as low as 120°C. A nonsilanized solid support made from diatomaceous earth or glass is basic in nature. We consider such supports

unsatisfactory at high temperature because they act as a weak base and cause degradation of the silicones. Silanization of the support reduces this degradation effect, permitting high temperature operation without rapid loss of silicone. When silane-treated supports are employed the upper temperature limits of operation approach the ideal limits as determined by thermogravimetric analysis (TGA).

THE NEW PHASES

The new silicones can all be classified according to the scheme used in the November, 1967, Newsletter. OV-101 and General Electric GC grade SE-30 are pure methyl silicones. The new OV-25 phase is 75% phenyl substituted; OV-225 is 25% phenyl, 25% cyanopropyl substituted; and OV-210 is 50% trifluoropropyl substituted. The temperature limits suggested are based either on actual GC studies or on TGA studies. To show graphically the differences in silicone stationary phases, we have prepared the following table which gives all the useful silicones. Except for the first two substitution classes listed below, which contain nonpolar phases, all the phases within a substitution class are listed in order of decreasing polarity.

METHYL SILICONES (NONPOLAR)

	Suggested Temp. Limits°C	Solvent	Physical Character
OV-1	350+	Chloroform (1)	Gum
OV-101	350+	Toluene	Oil
GE SE-30 (GC Grade)	350+	Chloroform	Gum
JXR	300	Toluene	Gum
DC 410	300+	Acetone	Gum
UC L-45	300	Toluene	Oil
Reg. GE SE-30	300	Chloroform	Gum
GE SF-96	250	Toluene	Oil
DC 200	250	Toluene	Oil

VINYL SUBSTITUTED (NONPOLAR)

	Suggested Temp. Limits°C	%Substituted	Solvent	Physical Character
UC W-98	300+	Unknown	Toluene	Gum
DC 430	300	Unknown	Toluene	Oil

PHENYL SUBSTITUTED (MODERATELY POLAR)

	Suggested Temp. Limits°C	%Substituted	Solvent	Physical Character
OV-25	300+	75%	Chloroform	Gum
OV-17	350+	50%	Acetone	Oil
DC 710	225+ (2)	50%	Acetone	Oil
DC 550	225+ (2)	25%	Toluene	Oil
DC 560	300	5% (3)	Acetone	Oil
GE SE-54 & 52	300	5% (4)	Chloroform	Gum

HALOGEN SUBSTITUTED (MODERATELY POLAR)

	Suggested Temp. Limits°C	%Substituted	Solvent	Physical Character
OV-210	275+ (5)	50% Trifluoropropyl	Acetone	Oil
DC QF-1	250	50% Trifluoropropyl	Acetone	Oil
GE Versilube F-50	300	10% Trichlorophenyl	Toluene	Oil

NITRILE SUBSTITUTED (POLAR)

	Suggested Temp. Limits°C	%Substituted	Solvent	Physical Character
GE XF-1150	200	50% Cyanoethyl	Acetone	Oil
OV-225	250+ (5)	25% Cyanopropyl 25% Phenyl	Acetone	Oil
GE XE-60	250	25% Cyanoethyl	Acetone	Gum
GE XF-1105	200	5% Cyanoethyl	Acetone	Oil

- (1) Maximum solubility 10g/100 cc.
- (2) These limits not well defined.
- (3) Estimated by IR.
- (4) SE-54 also contains about 1% vinyl.
- (5) Maximum limit under actual use with FID.

We will first demonstrate the similarity of the non-selective phases OV-101, DC-200, and SE-30. We will then attempt to illustrate the application of selectivity rules to some simple pesticide separations. The elution order of 7 important and common pesticides is shown in Table I. The elution order is expressed by Relative Retention of the pesticides to Aldrin since this is common accepted practice. The table also serves to illustrate the retardation effect of OV-17.

Conclusions can be drawn by a mere cursory examination of Table I.

- 1 DC-200 and OV-101 exhibit the same separating characteristics.
- 2 SE-30 differs from DC-200 and OV-101 in its retention of DDT.

Otherwise it is closely similar. We might attribute this difference to an effect of molecular weight of stationary phase. The three phases are identical in structure and differ only in molecular weight.

- 3 OV-17 retards benzene and naphthalene type pesticides. Remember how OV-17 retarded benzene from cyclohexane? OV-17 is displaying its "phase affinity" for both the benzene and the naphthalene rings (Dieldrin and Endrin).

A more striking illustration of OV-17's phase affinity for benzene as compared to DC-200 is shown in Table II. Here we list the DDT relatives, all of which have benzene rings inherent in their structure.

TABLE II

	9% OV-17	10% DC-200	12% QF-1
Aldrin	1.00	1.00	1.00
op DDE	1.94	1.47	1.44
pp 'DDE	2.39	1.81	1.88
op TDE	2.83	1.87	2.25
pp 'TDE	3.60	2.31	3.20
op DDT	3.42	2.41	2.33
pp 'DDT	4.44	3.07	2.47

Again conclusions may be made about phase behavior:

- 1 OV-17 retards all DDT type compounds.
- 2 QF-1 shows a variable selectivity toward the DDT relatives. It retards some and accelerates others. This is not "phase affinity" for benzene, but rather a complex selectivity for structure other than benzene.

Using the variable "selectivity" behavior of QF-1 and OV-17's "phase affinity", we prepared a mixed phase packing which would separate the 12 different pesticides listed in Tables I and II.

TABLE I
RELATIVE RETENTION TIME (ALDRIN = 1)

Pesticide	10% DC-200 Pest. Manual	5% OV-101 Appl. Sci.	10% SE-30 Appl. Sci.	9% OV-17 Appl. Sci.
Lindane	0.47	0.46	0.47	0.63
Heptachlor	0.79	0.79	0.80	0.80
Aldrin	1.00	1.00	1.00	1.00
Heptachlor Epoxide	1.24	1.24	1.22	1.50
Dieldrin	1.83	1.83	1.79	2.35
Endrin	2.05	2.05	2.01	2.93
pp 'DDT	3.03	3.04	2.88	4.44

The QF-1, OV-17, or DC-200 columns alone would not do all 12. The results are shown in a Technical Paper on the next page. Such a mixed phase column is used for rough screening of pesticide samples because of its great resolving power.

In order to keep all the available relative retention data in one section, we include Food & Drug Administration's Pesticide Manual excerpts immediately after the Mixed-Phase paper.*

*Retention Data from the other mixed phase packing mentioned previously may be found in Burke & Holswade. JAOAC 49, 374 (1966), or in Section 311.58 of Food & Drug Administration's Pesticide Manual, Volume I of 1/1/68. You should have all this retention data at your fingertips.

We cannot develop extensive relative retention data for pesticides. Facilities are limited, time is always short, and the cost is prohibitive. Insufficient data exists to permit easy column selection for pesticide analysis.

You, the Pesticide Analyzers, are in the best position to develop the needed data. It would be ideal if the relative retention time data were collected for all pesticides listed in the following table on at least 3 other columns. We suggest: 10% QF-1, 10% OV-17, and 5% OV-225 columns be used for such a study. These three phases exhibit quite different selectivities and the data would simplify column selection immeasurably.

If you choose to help accumulate this data, beware of a dangerous pitfall: THINK before you report, and check the results based on some considerations of pesticide structure. See APPENDIX I.

Relative Retention Time and Response Table
10% DC 200 ON GAS-CHROM Q (OR ANAKROM ABS)

Column	Glass, 6ft x 4 mm i. d. ; 10% DC 200 (12,500 est) on 80/100 mesh Gas-Chrom Q (or 80/90 mesh Anakrom ABS)
Column temperature	200°C
Injection temperature	225°C
Carrier gas flow rate	120 ml/min N ₂
Detector	Tritium source; concentric type; operated in electron capture mode
Detector temperature	200°C
Detector voltage	DC voltage at which 1 ng of heptachlor epoxide causes 1/2 full scale recorder deflection at 1×10^{-9} AFS, attenuator 1
Recorder	5 millivolt
Electrometer sensitivity	1×10^{-9} AFS, attenuator 1

Pesticide	Retention Time Relative to Aldrin Ratio	Response ^a ng for 1/2 FSD @ 1×10^{-9} AFS
CDAA	0.1	4
DDVP	0.1	10
Monuron	0.1	30
Diuron	0.11	10- 15
Neburon	0.11	10- 15
Hexachlorocyclopentadiene	0.12	0.4
Phosdrin	0.13	18
Simazine	0.18, 0.40	100- 300
Propazine	0.18, 0.42	100- 300
Atrazine	0.18, 0.42, 1.71, 2.18, 5.0	100- 300
TEPP	0.21, 0.89, 2.95	3500-4500
TCNB	0.28	0.5-0.6
Methyl ester 2, 4-D	0.29	10
CIPC	0.31	1000-2000
Thimet**	0.35	20- 30
Vegadex	0.37	3
BHC	0.38, 0.47	1- 2
BHC (alpha)	0.38	0.4
Dimethoate	0.40	4.5
Isopropyl ester 2, 4-D	0.41	10
BHC (beta)	0.42	1.75
Hexachlorobenzene	0.43	0.3-0.4
Methoxychlor (tech)	0.46, 1.64, 4.5	8- 10
Chlordene	0.55	0.6
Lindane (gamma BHC)	0.47	0.5
PCNB	0.50	0.3-0.4
Diazinon**	0.50	20- 25
Dichlone	0.54	3- 4
Tetraiodoethylene**	0.54	4
Isobutyl ester 2, 4-D	0.61	5
Endosulfan alcohol (a)	0.63, 1.28, 1.64, 2.07	14

Relative Retention Time and Response Table (Continued)

10% DC 200 ON GAS-CHROM Q (OR ANAKROM ABS)

Pesticide	Retention Time Relative to Aldrin Ratio	Response* ng for 1/2 FSD @ 1×10^{-9} AFS
Isopropyl ester 2, 4, 5-T	0.66	2
Methyl parathion**	0.68	2- 3
n-Butyl ester 2, 4-D	0.71	40
Ronnel	0.78	1
Dimite	0.78, .95	15-20
Heptachlor	0.79	0.75-1
Malathion**	0.87	20-30
1-Hydroxychlorodene	0.97	1.3
Parathion**	0.98	15
Dacthal	0.99	1.2
Aldrin**	1.00	0.75-1
Kelthane	1.01	2- 3
Chlorothion	1.05	60-70
Chloropropylate	1.06, 1.75, 2.32	60-70
Chlorobenzilate	1.06, 1.63, 2.28	60-70
n-Butyl ester 2, 4, 5-T	1.08	2
Telodrin	1.10	1
Chloranil	1.11, 1.28	50
Isodrin	1.15	1
o, p'-TDE olefin	1.17	16
Folpet	1.20	4
Dyrene	1.21	75
Captan	1.22	40-50
Sulphenone	1.24	4
Heptachlor epoxide	1.24	1
o, p'-Dichlorodiphenyl- monochloroethane	1.34	-
Chlorbenside	1.36	2
p, p'-TDE olefin	1.42	2- 3
Chlordane (alpha)	1.43	1
Perthane olefin	1.47	40
o, p'-DDE	1.48	2
Propylene glycol butyl ether ester, 2, 4-D	1.51	20
Endosulfan	1.58, 2.16	1- 2
Endosulfan I	1.58	1.5
Ovex	1.58	3
Chlordane (beta)	1.60	1
p, p'-Dichlorodiphenyl- monochloroethane	1.62	-
Delta Trichloro "237" (b)	1.67	2
Butoxy ethanol ester 2, 4-D	1.78	12
DDT (tech)	1.81, 2.44, 3.03	5
p, p'-DDE	1.81	1
Dieldrin	1.83	1-1.5
o, p'-TDE	1.86	2
Iso-octyl ester 2, 4-D	2.00, 2.37	30

Relative Retention Time and Response Table (Continued)
10% DC 200 ON GAS-CHROM Q (OR ANAKROM ABS)

Pesticide	Retention Time Relative to Aldrin Ratio	Response ^a ng for 1/2 FSD @ 1×10^{-9} AFS
Endrin	2.05, 2.29	2
Ethyl hexyl ester 2, 4-D	2.07	8
Perthane	2.11	50-150
Endosulfan II	2.16	2
p, p'-Methoxychlor, monochloro- ethylene analog (c)	2.22	35
Endrin Aldehyde (d)	2.30	4
o, p'-DDT	2.44	4
Ethion**	2.46	10- 12
Endrin alcohol (d)	2.50	3.5
Endosulfan sulfate (e)	2.67	5
Kepone	2.70	4- 5
p, p'-TDE	2.70	1.5
Iso-octyl ester 2, 4, 5-T	2.73, 3.14, 3.73	10
Carbophenothion	2.83	10
Prolan	2.83	5
Perthane, trichloroethane analog (f)	2.83	6
Dilan	2.83, 3.37	5- 10
Butoxy ethanol ester 2, 4, 5-T	2.85	4
p, p'-Methoxychlor olefin	2.91	8
p, p'-DDT	3.03	4
Bulan	3.35	4
p, p'-Methoxychlor, dichloroethane analog (g)	3.36	250
Delta Keto "153" (d)	3.50	5
Imidan**	3.87	15
Methoxychlor	4.5	8- 10
Guthion**	5.0	50
Tetradifon	5.4	5- 7
BEP ester 2, 4, 5-T	0.16, 0.67, 1.06, 2.79 3.24, 5.2, 6.9	35
Chlordane (tech)	0.44, 0.62, 0.70, 0.79, 0.95, 1.14, 1.42, 1.59, 2.56	12
BEP ester 2, 4-D (tech)	0.73, 1.84, 2.10, 3.33, 4.9	50
Strobane	1.07, 1.29, 1.50, 1.76, 1.90, 2.05, 2.28, 2.64, 3.04, 3.55	25
Toxaphene	1.18, 1.51, 1.76, 2.34, 2.63, 3.06, 3.61, 4.3, 4.5, 5.0	20
Co-Ral	9.5	100-150

Relative Retention Time and Response Table (Continued)

10% DC 200 ON GAS-CHROM Q (OR ANAKROM ABS)

- (a) 1, 4, 5, 6, 7, 7 - hexachloro - 2, 3 - bis (hydroxymethyl) - bicyclo - [2, 2, 1] heptene - 5
- (b) "Ennechlor" or 1, 2, 3, 4, 5, 6, 7, 8, 8 - nonachloro - 2, 3, 3a, 4, 7, 7a - hexahydro - 4, 7 - methanoindene
- (c) 2, 2 - bis (p-methoxyphenyl) 1-chloroethene [Prepared by alkaline treatment of 2, 2-bis (p-methoxyphenyl) - 1, 1-dichloroethane]
- (d) Phillips, D.D., et al, J. Agr. Food Chem. 10, 217 (1962)
- (e) 6, 7, 8, 9, 10, 10 - hexachloro - 1, 5, 5a, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3 - benzodioxathiepin 3, 3 - dioxide
- (f) 2, 2 - bis (p-ethylphenyl) - 1, 1, 1 - trichloroethane. [Prepared per: Forrest, James, et al; J. Chem. Soc. 333-9 (1946)]
- (g) 2, 2 - bis (p-methoxyphenyl) - 1, 1 - dichloroethane. [Prepared per: U.S. patent #2, 484, 057, Oct. 11, 1949]

Table contains compounds that are not commercial pesticides themselves but which may be expected to result from use of certain pesticides (e.g., DDE).

When more than one peak is present the major peak (s) is underlined.

Retention times are measured from leading edge of solvent peak.

Chlorophenoxyacetic acids and their salts do not chromatograph.

*Quantity (nanograms) of pesticide chemicals required to cause 1/2 full scale recorder deflection at an electrometer sensitivity of 1×10^{-9} AFS when voltage is selected to give 1/2 scale deflection for heptachlor epoxide. The approximate response of the electron capture detector to chlorinated pesticide chemicals may vary among individual detectors. All detectors may not have the same response characteristics at given operational parameters, i.e., detector voltage and temperature and carrier gas flow rate.

The quantity of certain pesticides necessary for a 1/2 scale recorder deflection is also dependent on the "condition" of the GLC column. This is the case of endrin, perthane, Kelthane, tetradifon, methoxychlor, heptachlor, DDT, malathion, Guthion, and possibly others.

The response data reported here was obtained on columns believed to be well conditioned.

**Not chlorinated.

***Aldrin reference. Retention time approximately 4.25 minutes.

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- 1 Burke, J., and Giuffrida, L., JAOAC, 47, 326 (1964)
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COLUMN PACKING
MIXED-PHASE SILICONE PACKING FOR PESTICIDE
ANALYSIS BY GAS CHROMATOGRAPHY

Twelve chlorinated pesticides were almost completely separated by use of a mixed packing prepared from QF-1 and new phenylmethyl silicone.

Retention data for pesticides on a mixed-phase packing were recently reported by Burke and Holswade (1). The packing consisted of equal portions of 15 weight % QF-1 on 80/100 Gas Chrom Q and 10 weight % DC 200 (12,500 cs.) on 80/100 Gas Chrom Q made separately and then thoroughly mixed together. The purpose of the work by Burke and Holswade was to develop a packing with a polarity different from that of the dimethyl silicones (such as DC 200 or SE-30), which would separate a large number of chlorinated pesticides. The present work consists of comparing mixed and individual phases and was prompted by the availability of a new phenylmethyl silicone, OV-17. The results indicate almost complete separation of 12 chlorinated pesticides: lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, p,p'-DDT, o,p'-DDT, p,p'-DDE, o, p'-DDE, p,p'-TDE, and o,p'-TDE.

EXPERIMENTAL

The chromatograph used was a Barber-Colman 5000 equipped with both flame and electron-capture detectors. The columns were 6 foot $\times$ 4 mm. i.d. glass U-tubes, filled with packings prepared with 80/100 and 100/120 Gas Chrom Q and one or more of the following stationary phases: (1) QF-1 (also known as FS-1265), (2) DC 200 (12,500 cs.), and (3) OV-17, a phenylmethyl silicone.

The packings were prepared by the fluidization technique described by Kruppa (2). In the case of mixed-phase packings, the individual packings were first prepared and then subsequently mixed thoroughly in the proper proportions.

RESULTS

The authors' results agreed well with those of Burke and Holswade on the QF-1 + DC 200 packing for the 12 pesticides (Burke and Holswade presented retention data for many more pesticides). The results from the OV-17 packing were different from those of the other packings, but except possibly for special applications, did not seem significantly important. All the packings tested gave separation factors for two or more pairs of pesticides which were too small to result in any appreciable separation. However, the retention data from the OV-17 and the QF-1 packings suggested that the proper mixture of these two packings might separate all 12 pesticides.

The best results were obtained from a mixture of equal portions of 7 weight % OV-17 and 9 weight % QF-1 on 100/120 Gas Chrom Q. Whether this is the optimum combination for phase loading has not yet been determined. However, all but two of the 12 pesticides were almost completely separated and these two (p,p'-DDE and dieldrin) were partially separated. If the column efficiency can be improved (it was 2000 vs. the usual 3000 to 4000 theoretical plates obtained with DC 200), the separation would be appreciably improved.

A chromatogram of the 12 pesticides is shown in Figure 1. Retention times relative to aldrin are presented in Table I for five packings. Separation factors for adjacent pesticides are shown in Table II for the OV-17 + QF-1 and DC 200 + QF-1 mixed packings.

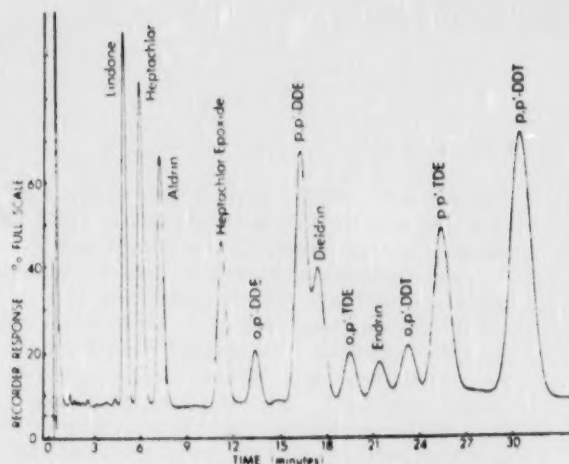


Figure 1. Chromatogram of 12 pesticides

Equal parts by weight of 7 weight % OV-17 and 9 weight % QF-1 on 100/120 Gas-Chrom Q at 200° C. and 30 p.s.i.g.
Column: 6-foot X 4-mm. i.d. glass U-tube
Electron-capture detector

Table I. Retention Times Relative to Aldrin

	OV-17 ^a + QF-1	OV-17 ^b	QF-1 ^c	DC 200 ^d	DC 200 ^e + QF-1
Lindane	0.66	0.63	0.77	0.48	0.58
Heptachlor	0.81	0.80	0.77	0.79	0.81
Aldrin	1.00	1.00	1.00	1.00	1.00
Heptachlor epoxide	1.57	1.50	1.80	1.23	1.46
o,p'-DDE	1.89	1.94	1.44	1.47	1.47
p,p'-DDE	2.30	2.39	1.88	1.81	1.88
Dieldrin	2.47	2.35	2.73	1.82	2.22
o,p'-TDE	2.77	2.83	2.25	1.87	2.04
Endrin	3.05	2.93	3.22	2.04	2.50
p,p'-TDE	3.62	3.60	3.20	2.31	2.70
o,p'-DDT	3.30	3.42	2.35	2.41	2.48
p,p'-DDT	4.36	4.44	2.42	3.07	3.28

^a Equal parts of 7% OV-17 + 9% QF-1 on 100/120 Gas Chrom Q at 200° C. and 20 p.s.i.g. Absolute retention time for aldrin, 7.12 min.

^b 9% OV-17 on 100/120 Gas Chrom Q at 205° C. and 40 p.s.i.g. Absolute retention time for aldrin 12.11 min.

^c 12% QF-1 on 100/120 Gas Chrom Q at 200° C. and 35 p.s.i.g. Absolute retention time for aldrin 2.01 min.

^d 10% DC 200 on 100/120 Gas Chrom Q at 200° C. and 30 p.s.i.g. Absolute retention time for aldrin 6.11 min.

^e Equal parts of 10% DC 200 + 15% QF-1 on 80/100 Gas Chrom Q at 200° C. and 30 p.s.i.g. Absolute retention time for aldrin 8.35 min.

^f Not calculated. Heptachlor very close to lindane.

Table II. Separation Factors between Adjacent Pesticides

OV-17 + QF-1		DC 200 + QF-1	
Lindane	1.23	Lindane	1.40
Heptachlor	1.24	Heptachlor	1.23
Aldrin	1.57	Aldrin	1.46
Heptachlor epoxide	1.20	Heptachlor epoxide	1.01
o,p'-DDE	1.22	o,p'-DDE	1.28
p,p'-DDE	1.08	p,p'-DDE	1.08
Dieldrin	1.12	Dieldrin	1.09
o,p'-TDE	1.10	o,p'-TDE	1.12
Endrin	1.09	Endrin	1.03
o,p'-DDT	1.10	o,p'-DDT	1.06
p,p'-DDT	1.20	p,p'-DDT	1.22

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PREPARATION OF COATED PACKINGS

Use of the Hi-Eff Fluidizer*

I DESCRIPTION

If a gas is passed upward through a porous plate covered with a finely divided solid, the particles are suspended and intimately mixed in the gas stream. The suspended particles exhibit fluid characteristics and the process is called fluidization.

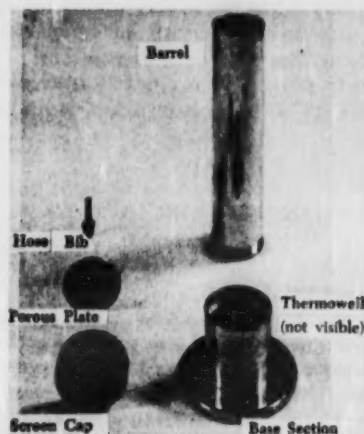
The basic parts of the Hi-Eff Fluidizer are shown and labeled in the exploded view. The base section, cap and barrel are made of chrome-plated brass. The screen cap prevents the packing from blowing out of the barrel if the gas rate is initially too high. The barrel screws into the base section and clamps the porous plate in position. The base is designed with a spiral insert (silver soldered in place) which provides good heat transfer characteristics for preheating the gas when the fluidizer is set on a hot plate. The base also contains a 6mm thermowell to permit measurement of the gas temperature. A hose bib and a compression fitting, which are interchangeable, are included with the unit, permitting the use of either metal or rubber tubing to connect the fluidizer to a gas cylinder.

The unit is already assembled when received but can be stripped down to its basic parts simply by unscrewing the barrel from the base, exposing the porous bronze plate. The plate rests on a flange and drops out when the base is inverted.

II CLEANING

The unit can be simply cleaned by blowing out residual packing with compressed gas, but to thoroughly clean the fluidizer it should be disassembled and the barrel, cap and porous plate soaked in a suitable solvent. The base

section needs no cleaning unless a solution of phase has been inadvertently poured into the unit without gas flow. In this case, clean the base section with the solvent used for the phase. **WARNING: IF THIS HAPPENS WHEN THE BASE IS HEATED, THE BASE CAN BECOME PLUGGED AND THE POROUS PLATE RUINED.**



III GASES

Nitrogen is recommended for the fluidization gas because it is inert and inexpensive although compressed air can be used for phases which are not sensitive to oxidation. A Molecular Sieve or Drierite trap can be inserted in the gas line between the cylinder and the fluidizer if desired. A cylinder reducer valve for the 0-50 psig range is recommended, although a 0-250 psig value may be used if great care is taken when turning on the fluidizing gas.

* Patent Applied For

IV PRECAUTIONS

This unit may be used to prepare almost every packing used for gas chromatography columns EXCEPT: highly corrosive materials such as silver nitrate, strong acids (H_3PO_4 , H_2SO_4 , etc.), strong bases ($NaOH$, KOH , etc.), or CORROSIVE ORGANICS. Teflon supports have not been studied.

There is no need to operate this unit above $150^\circ C$ which is its temperature limit. Discoloration of the chrome plating on the base can be expected from prolonged usage or excessive temperature exposure but this will in no way interfere with the operation of the unit. If the barrel becomes badly discolored it should be replaced, although this is unlikely if the temperature limit is observed.

V GENERAL OPERATION

A small amount of GAS-CHROM S (50 g) is supplied with the Hi-Eff Fluidizer. To become familiar with the operation of this unit, it is suggested that the user practice with this support by carrying the support through the filtration procedure (Section VII) with solvent alone (a stationary phase is not necessary in this case). Use of the fluidizer is limited to a maximum of 50 grams of packing. Heat is supplied to the packing from the preheated gas by setting the fluidizer on a hot plate (any type available in the laboratory is satisfactory). Heat from the hot plate is transferred to the gas which flows through a long spiral path in the base and emerges below the porous plate. Gas temperature can be measured by inserting a laboratory thermometer in the well in the base. The heat supply should be controlled by operating the hot plate through a laboratory Variac which in turn will control the gas temperature below the porous plate within a $5^\circ C$ range.

In general, good packings can be made with gas temperatures in the range of 40 to $120^\circ C$. A good rule of thumb is to operate with the

gas temperature just below the boiling point of the solvent being removed. With solid stationary phases, best results appear to be obtained with gas temperatures below the melting point of the phase. THE FLUIDIZER WILL OPERATE CORRECTLY WITH GAS PRESSURES OF 1 TO 5 PSIG.

VI TEMPERATURE CALIBRATION

Although the gas temperature can be measured while drying a packing, it is recommended that a simple temperature-versus-Variac voltage calibration be done initially with user's hot plate. This allows the voltage to be preset when a packing is to be dried.

To carry out this calibration, place about 25 grams of dry support (as supplied) in the fluidizer, place the fluidizer on the hot plate and apply a low heat (about 20 volts Variac setting). After 10 minutes gradually start the gas flow and set a rate which will result in gentle fluidization of the support. (See Notes 1 and 2.) Take successive temperature readings at 5 minute intervals until two consecutive readings agree within 5 degrees. Record this temperature and voltage. Raise the voltage in several increments until a gas temperature of $120^\circ C$ is reached, record the temperature for each voltage setting and make a plot of gas temperature versus Variac voltage.

Use of this plot without measuring temperatures while drying a packing should be satisfactory. However, if desired, the gas temperature can be measured during operation and a finer control of temperature obtained by small adjustments of voltage. (REMEMBER: DO NOT EXCEED $150^\circ C$.)

VII FILTRATION-FLUIDIZATION PROCEDURE

The following symbols will be used in this description:

x = weight of support to be coated

y = w/v % stationary phase divided by 100

- Step 1. Weigh x grams of support into a beaker or similar container. Weigh $5xy$ grams of stationary phase in another beaker (Note 3). Measure $5x$ ml of solvent in a graduated cylinder and add to the stationary phase (Note 4). Completely dissolve the liquid phase in the solvent by stirring with a glass rod or with a magnetic stirrer. Minimize evaporation of the solvent so as not to change stationary phase concentration significantly.
- Step 2. Place the Hi-Eff Fluidizer on the hot plate, turn on the variac and let the unit warm up without gas flow while performing Step 3. Step 3 and warm-up of the fluidizer both require about 10 minutes.
- Step 3. Slowly add the support to the solution with constant but gentle stirring with a glass rod or other convenient device. Let the support settle for 5 minutes (Note 5), then slurry again by gentle stirring (Note 6). Immediately pour the cream-like mixture into a Buchner funnel mounted on a vacuum filter flask. Apply vacuum to the flask with an aspirator and filter off the excess solution. Continue filtration until dripping of the solution from the funnel ceases.
- Step 4. Transfer the damp packing to the fluidizer through a widemouth funnel by inverting the Buchner funnel over the fluidizer and tapping gently with the heel of the hand. Slowly turn on fluidizing gas and closely observe the packing. It will rise upward as a solid plug and the proper gas setting can be made by allowing $1/4$ to $1/2$ inch rise of the plug in the barrel (Note 1).
- Step 5. Put the cap in place and leave the unit alone for 2 minutes. After 2 minutes the packing should begin to fluidize. If the fluidization is too violent, decrease the gas flow rate (Note 2). The packing is dry when no odor of solvent can be detected in the effluent gas stream at the top of the fluidizer. The total drying time

is 5 to 15 minutes depending on the amount of packing and volatility of the solvent used in making the packing.

- Step 6. Pour the finished packing from the fluidizer by grasping the barrel near its top (Note 7). Set the fluidizer aside to cool and clean it with compressed gas or solvent as described under CLEANING (Section II).

VIII SLURRY FLUIDIZATION PROCEDURE

WARNING: IF THIS PROCEDURE IS TO BE USED, NEVER PUT PHASE SOLUTION INTO UNIT WITHOUT GAS FLOW.

The method suggested here is adapted from Kaiser (2) and appears to work quite well with this unit. This method may be used for heavy coats (above 10%) at the discretion of the user. For phase loadings above 20% this will prove the most economical and may give as good a packing as the filtration procedure does for that high a loading.

Gas (compressed air, nitrogen) is blown through the unit at a rate of about 6 cu. ft./hr. While gas is flowing, 25 to 50 g of the dry support is tipped into the unit. The calculated quantity of stationary phase, diluted with appropriate solvent is then added and gently stirred. The quantity of solvent used is calculated in such a way that at the start of the impregnation process the stationary phase solution completely covers the solid phase. The temperature is controlled in such a way that the whole process is complete within 30 minutes for 25 g of packing with chloroform and a gas inlet temperature of 60°C. "The wash gas must, of course, be pure and above all dry. This method gives a substantial yield of good quality column packings and is readily reproducible. As the material is subjected to hardly any mechanical stress, supports liable to abrasion may be treated."

IX NOTES

1. When gas flow is started, the gas pressure should be raised slowly and carefully. If too much pressure is suddenly applied, the slug of wet packing will blow out the top of the fluidizer.

2. If the gas rate is too high after fluidization of the packing starts, the packing will impinge on and cling to the underside of the screen cap. This is readily observed if it occurs. In this case, decrease the gas rate and remove the cap temporarily to observe the packing. When fluidization is correct, the packing will look like a gently boiling liquid. The gas rate can be adjusted accordingly but pressures in the range of 1 to 5 psig will give the desired results.

3. The fluidizer is limited to 50 gram charges. In most cases a $y\%$ (w/v) solution of stationary phase will result closely in a $y\%$ (w/w) coating on the packing. The most notable exceptions are the silicone gums such as OV-1, JXR, SE-30, W-98, etc. In general, a $y\%$ (w/w) solution of these silicones will make a $2y\%$ (w/w) coating. Thus a 1.5% (w/v) solution of OV-1 will produce a 3% (w/w) coat. Polyesters and organosilicone phases tend to coat slightly high at high phase loadings. Thus a 3% (w/v) solution will produce a 3% (w/w) coat, a 10% (w/v) solution will produce an 11% (w/w) packing, and a 16% (w/v) solution results in a packing of about 19% (w/w) coat.

The best method for determining phase concentration coated on the support is to perform a Soxhlet extraction on a portion of the packing. If this is done over a range of concentrations, a calibration curve can be produced. (3)

A simpler alternative, although not exact, is based on the solution retained by the packing during filtration. There is good indication that the amount of phase coated on the packing is directly proportional to the volume of solution remaining on the support after the filtration step.

Let F = the filtrate volume in ml.

then $5x - F = S$ ml solution retained on support

Then % Coat = $100Sy/(x+Sy)$

In this laboratory, the above method has been found to be quite accurate for large (i.e., 500g) batches of packings. The inaccuracies encountered with small scale batches depend on the evaporative losses of the solvent. Sample problems are shown on page 8.

4. For support mesh cuts of 80/100 or higher, a ratio of solvent to support (vol./wt.) of 5 is used. For mesh cuts below 80/100 such as 60/80, this ratio should be 6. Therefore, 6x ml of solvent and 6xy grams of stationary phase should be used for mesh cuts of 60/80 or below.
5. At the end of 4 minutes it is advisable to remove all particles which float on the surface of the solution. These particles are not wet by the solution and therefore are not coated. They can be skimmed off the surface with an eyedropper inserted into a piece of rubber vacuum tubing connected to the filter flask to be used for filtration of the packing. By applying a vacuum to the filter flask, the particles are drawn into the flask. Minimize the amount of solution removed and do not empty the filter flask if you plan to estimate the % coat by using the filtrate measurement technique described in Note 3.
6. When stirring, do not rub or abraid the packed-down support. Stir the solution vigorously, starting at the top of the support and working down slowly until all the support is slurried.
7. The base and lower part of the barrel become hot during normal operation. However, the top of the barrel should remain cool enough to hold unless the temperature limit is exceeded.

X OTHER DRYING TECHNIQUES

If the fluidizer is not to be used as the drying method, the basic procedures outlined in this Bulletin can still be followed. The other drying methods are well described in these references:

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Zubyk, W.J., Conner, A.Z. Anal. Chem. 36, 912. 1960.

Wotiz, H.H., Chattoraj, S.C. Anal. Chem. 36, 1467. 1964.

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SAMPLE CALCULATIONS

Sample 1

10% DC200 on 80/100 GAS-CHROM Q

$$\begin{aligned} x &= 25\text{g Support} \\ 5x &= 125\text{ ml CHCl}_3 \\ y &= \frac{10\%}{100} = 0.10\text{ g/ml} \end{aligned}$$

$$5xy = 125(0.10) = 12.5\text{g of DC200}$$

After Filtration

$$F = 95\text{ cc}$$

$$S = 5x - F = 125 - 95 = 30\text{ cc}$$

$$100 \frac{Sy}{x+Sy} = 100 \frac{(30)(0.10)}{25+(30)(0.10)} = 10.7\% \text{ Coat}$$

Sample 2

3% OV-1 on 100/120 GAS-CHROM Q

$$\begin{aligned} x &= 50\text{g Support} \\ 5x &= 250\text{ ml CHCl}_3 \\ y &= \frac{3\%}{100} = 0.03\% \end{aligned}$$

$$5xy = 250(0.03) = 3.75\text{g of OV-1}$$

After Filtration

$$F = 150\text{ ml recovered}$$

$$S = 5x - F = 250 - 150 = 100\text{ml}$$

$$100 \frac{Sy}{x+Sy} = 100 \frac{(100)(0.03)}{50+(100)(0.03)} = 2.9\% \text{ Coat}$$

Sample 3

15% EGSS-X on 60/80 GAS-CHROM P

$$\begin{aligned} x &= 25\text{g Support} \\ 6x &= 150\text{ ml CHCl}_3 \\ y &= \frac{15\%}{100} = 0.15 \end{aligned}$$

$$6xy = 150(0.15) = 19.5\text{g EGSS-X}$$

After Filtration

$$F = 115$$

$$S = 6x - F = 150 - 115 = 35\text{ml}$$

$$100 \frac{Sy}{x+Sy} = 100 \frac{(35)(0.15)}{25+(35)(0.15)} = 15.4\% \text{ Coat}$$

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- 2 Kaiser, Rudolf. Gas Chromatography, Vol. 1, p. 56. Butterworths, Washington D.C. 1963.
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PREPARATION OF COLUMN PACKINGS*

The primary goal in the packing preparation is uniform phase distribution over the surface of the solid support. Ease of column conditioning, column efficiency, and pesticide response are all tied to the uniformity of the coating. Both methods for coating, slurry, and filtration will achieve a uniform coat only when the packing is dried uniformly with intimate mixing. A secondary goal is to minimize particle breakage during the coating and drying steps. The third goal is to eliminate the finer "dust" particles produced during the preparation of the packing. Regardless of the care taken, some fines are made during the packing preparation steps. These three goals should be kept in mind when preparing column packings.

The procedure for preparing column packings described in Bulletin 2A on the next few pages was demonstrated during the discussion.**

*See Appendix III for an excerpt from Food & Drug Administration's Pesticide Manual, Volume 1, 1968, for their method of packing preparation, column packing and conditioning.

**See Appendix II for Kruppa, Henly and Smead, Anal. Chem. 39, 851 (1967).

PREPARING AND PACKING THE COLUMNS

I INTRODUCTION

After carefully preparing an excellent packing, it would indeed be a shame to ruin its performance by ignoring the column preparation. There are as many ways to pack columns as there are column shapes. Since most pesticide work is done with U-shaped glass columns (usually 6 foot in overall length) the method of preparing these will be demonstrated and described in detail.

The primary objective in packing any GC column is to achieve a firm, uniform cylinder of packing with a minimum of particle damage. It is my opinion that merely pencil tapping the column provides insufficient settling force to create the firm uniform cylinder. The procedure used by Applied Science has been employed for 6 years and has stood the test of time. It does insure a firm, uniform cylinder without signs of excess breakage. We call it the Vibration Technique.

II CLEANING

The glass U-Tube column as received usually contains some oils and other contaminants from the drawing process used to form the tubing. Some people urge cleaning with chromic acid solution, but this shouldn't be necessary. To remove these oils, we employ repeated chloroform and acetone rinses passing about 300 ml of each solvent through the column. This is done with an aspirator attached to one end of the column. The solvent (first, chloroform) is poured into the open end of the U-Tube, then passes through the column and into the aspirator. The column, when rinsed completely, may be dried by continuing vacuum and blocking the inlet with any convenient thumb.

III SILANE TREATING

A fresh 10% Vol/Vol solution of DMCS in toluene is prepared. The solution is poured into the dry U-Tube column filling both legs to the top. Permit the column to stand upright for 10 minutes. The solution may then be poured out and the column rinsed with 300 ml of toluene added to the column continuously while vacuum is applied to one end. The column is disconnected from vacuum and filled with methanol at once. The methanol should be poured out and a second filling made. This should sit quietly for 5 minutes. Then the column is drained, connected to the vacuum again, and rinsed with 50-100 ml of methanol. The final rinsing should be neutral to pH paper. An acetone rinse will permit drying the column as described under Cleaning. The dried column is ready for packing. If humidity in the lab is high, it is preferable not to store the column, but rather pack it immediately.

IV VIBRATION TECHNIQUE

A small funnel with a short piece of rubber tubing attached to the tip is placed on the outlet leg of the upright column. Sufficient packing is added slowly to fill the base of the U and 3 inches up the leg from the bottom. A hand vibrator (ideal for this purpose) or other such device is used to vibrate the column to settle the packing. Close observation is necessary during the vibration to insure that settling occurs. When settling ceases, 2-5 seconds, vibration is stopped and packing added in 6 inch increments. Vibration is employed again to settle the packing. By repeating these steps the column can be rapidly and well packed. The last 1/4 inch of the column should be left

unpacked. The funnel is moved to the inlet leg and the procedure of adding 6 inch increments of packing followed by vibration repeated until the packing level is within 1-1/2 inches of the top (varies with column and instrument).

Slight additions are made to the outlet if necessary and a 1/4 inch glass wool plug is firmly seated in the outlet side. Trim off excess glass wool with scissors. The inlet does not need a glass wool plug if the column is kept upright and repeated installation and removal of the column is not anticipated. If a plug is used on the inlet, make it as small as possible.

V COLUMN CONDITIONING

Column conditioning is probably the simplest of all GC operations. For all silicone coated packed columns, the procedure is basically the same. At about 3 or 4:00 P.M. connect the column to the inlet of the chromatograph leaving the outlet disconnected. Set temperature to the suggested upper temperature limit of the silicone or 50°C hotter than intended operating temperature, whichever is lower. Turn gas on slowly to get about 40-80 ml/min flow. Let the column stay at these conditions overnight, or better yet, over a weekend.

We test our columns after 16 hours of conditioning with 1 µl of a standard mixture composed of high purity Lindane, Heptachlor, Aldrin, Heptachlor epoxide, Dieldrin, Endrin, and pp-DDT. The concentration of each component is 1 ng per µl of solution. We evaluate column performance (actually the performance of GAS-CHROM Q) by measuring the peak height ratios of Endrin to Dieldrin (E/D) and DDT to Dieldrin (DDT/D). The present standard for acceptance of GAS-CHROM Q is an E/D ratio of no less than 0.70 and a DDT/D ratio no less than 0.40. The column should be ready for use for actual analysis work on most pesticides after this minimum conditioning. We suggest you use these as your standards of column acceptance.

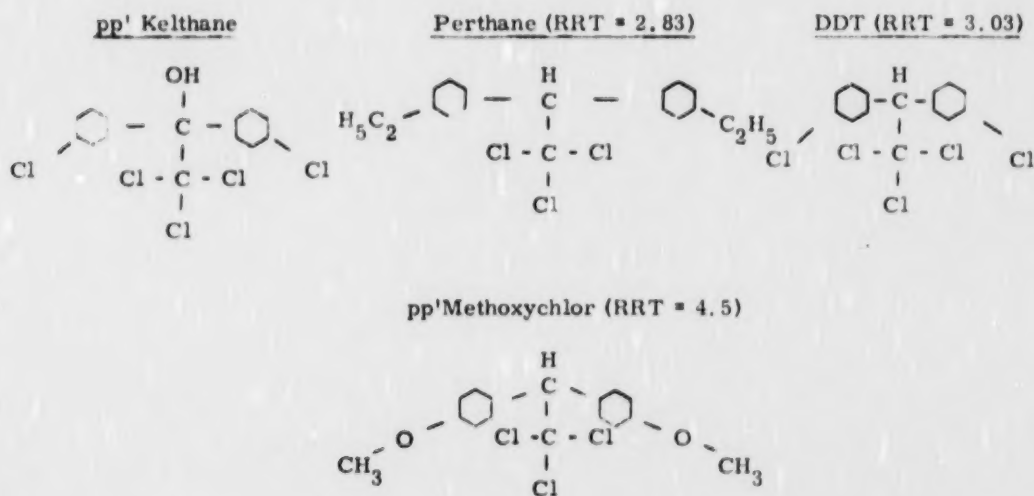
For the troublesome pesticides like Sevin, Kelthane, Perthane, etc., further conditioning may well be needed. In cases where decomposition or irreversible adsorption is encountered with these or other pesticides, repeated (5-6) heavy (5-10 µg each) injections of crude pesticides or plant extracts will usually do the trick. Spread these injections over the work day and let the column condition overnight. Check it for performance the next morning and repeat this harsh treatment if still unsatisfactory. These treatments should be done at conditioning temperature with the column disconnected from the detector. If the column is so recalcitrant that 4 days of such treatment does not work, make a new one! This treatment should not be necessary for any of the pesticides in the test mixture.

APPENDIX I

The value for relative retention time of Kelthane reported in Relative Retention Time and Response Table illustrates a failure to consider structure of a pesticide.

Kelthane was assigned a relative retention of 1.01 on a 10% DC-200 column.

This meant that the pesticide came off a DC-200 column at the same time as Aldrin. Compare the structure of Kelthane to other DDT relatives. We show the relative retention time to Aldrin of other DDT type compounds.



We studied Kelthane because of the obvious incongruity of its reported RRT.

We found:

1. Before the perfection of GAS-CHROM Q, Kelthane, on an average 10% DC-200 column, decomposed almost instantaneously giving one peak at a RRT of 1.00.
2. Kelthane is sensitive to traces of acid or base and decomposes with great ease.
3. By careful conditioning of a good DC-200 column prepared from GAS-CHROM Q we got a single peak for Kelthane with a Relative Retention Time of 4.40.

18-30

Pages ~~18-30~~ thru 18-34 purposely deleted.

APPENDIX III

302

TECHNIQUES AND GENERAL METHODS

302.1 Columns

302.11 Column Types

302.11a Aluminum, 6' x 4.5 mm i.d.
For microcoulometric gas chromatograph.

302.11b Pyrex glass, U-shaped, straight or coiled; 5-6' and 4-6 mm i.d., generally 6' x 4 mm i.d. For electron capture and thermionic equipped gas chromatographs.

Some analysts prefer silanized glass columns and silanized glass wool (used to plug ends of columns). See 122.1 (4) for reagents. Buy glass wool already silanized or silanize wool and columns by treating with 10% dimethyldichlorosilane in toluene for 10 minutes. Rinse the columns and wool with toluene and treat for 10 minutes with anhydrous methanol. Finally, rinse with methanol and allow to air dry.

302.12 Preparation of Column Packing.
Take care at all stages of column preparation to prevent fracturing the solid support.

302.12a Single Packing. Four methods of preparing packing are given below. Directions are for a 10% DC 200 column. Vary weight of coating and support for columns of other composition. These procedures provide material for two 6 foot columns.

(1) Coating by vacuum rotation (Division of Food Chemistry)

Weigh 2.0 g of DC 200 (Dow Corning Silicone Fluid 12, 500 CST) into a beaker. Dissolve in chloroform and transfer to a 300 ml 24/40 $\frac{5}{8}$ Morton-type round bottom flask, using in all about 100 ml of chloroform to dissolve and transfer. Add 18 g of support to flask, swirl to mix, and then let stand about 10 minutes. Place the flask on a rotary evaporator and rotate intermittently for several minutes

before applying heat (water bath) and slight vacuum. With the temperature of the bath at about 50°C, remove the solvent slowly, using a slight vacuum, and rotating intermittently. When the coated support appears damp, increase vacuum; when it appears dry, leave under vacuum to dry or transfer it to a shallow tray and allow to finish drying in air. Use only material that flows freely. Do not use material that clings to the wall of the flask.

(2) Coating by vacuum rotation (Philadelphia District)

Place 90 ml methylene chloride containing 2.2 g of DC 200 into a 300 ml 24/40 $\frac{5}{8}$ Morton-type round bottom flask and add 20 g of support. Attach to a rotary evaporator and rotate for 5 minutes. Put a T tube in the line of the vacuum connection and equip the side limb with a short rubber tube and open screw clamp. Rotate in a 30°C water bath and cautiously adjust the screw clamp, as required, to maintain boiling of the solvent. When the coated support appears just damp discontinue the vacuum and rotation. Place the flask in a bath at ca 50°C and rotate with increased vacuum, stopping when apparently dry. Loosen material from walls by tapping on a padded surface and air dry in a shallow pan.

(3) Filtration Coating Procedure. (Based on filtration and pressure packing techniques of Horning, et al, Methods of Biochemical Analysis, Ed. by D. Glick, XI, Interscience Publishers, New York, 1963).

Fill a 1 L vacuum flask half full with CCl_4 . Slowly pour 60 g of support onto surface of liquid. Stopper with a one-hole stopper. Apply reduced pressure (control vacuum with thumb over stopper) for

5 minutes with intermittent swirling. Let stand 5 minutes longer and decant fines. Add more CCl_4 and repeat this operation until few fines are being decanted. Dry to apparent dryness in a flat-bottomed dish on a steam bath in the hood and then in a convection type oven with minimum vibration for one hour at 130°C . (Removal of fines may or may not be necessary depending on the support used, the mesh cut involved and the state in which the support is received. If fines are removed the pressure packing technique will not lead to a too tightly packed column. It has been found that Gas Chrom Q does not usually require this treatment.)

Prepare a solution of the desired % stationary phase (w/v) by weighing the phase into a beaker, dissolving and transferring to a 100 ml graduated cylinder with hot toluene. Cool and make to volume. Place the solution into a liter side-arm vacuum flask, add about 20 g support, stopper with a one-hole stopper and reduce pressure (control with thumb as before) with water-pump aspirator for 5 minutes, with occasional swirling. Return to atmospheric pressure and let stand for 5 minutes. With rapid swirling motion, pour contents on 4" medium porosity sintered-glass funnel and then filter off excess liquid by reducing pressure with water-pump aspirator for 5 minutes. Release vacuum, invert funnel over flat-bottomed dish, gently transfer contents to dish and spread out to air dry for 15 minutes. Place in a convection oven at 130°C and dry for 1 hour.

- (4) Pan Method, (Hopkins, B.F., Dechert, D.D., and Martin, R.J., private communication, 1966).

In a 400 ml beaker dissolve 4.0 g DC 200 in 250 ml ethyl acetate (heating if necessary). Add 36.0 g Gas Chrom Q and allow to stand for 10 minutes with occasional gentle stirring. Transfer material to a clean stainless steel flat pan. Rinse the beaker with a small portion of ethyl acetate. Place the pan in a hood and allow the ethyl acetate to evaporate spontaneously. Frequently mix the material by gently tipping the pan back and forth. When

material becomes a slurry, continue to mix until the material ceases to flow. Allow to air dry, followed by 1 hour in 100°C oven. The material is ready to pack into the column.

302.12b Mixed Packing. To prepare a column packing of 15% QF-1 - 10% DC 200, use procedure 302.12a to apply 15% QF-1 by weight and 10% DC 200 by weight to separate portions of 80/100 mesh Gas-Chrom Q. Mix together in a flask equal weights of each coated support. Form a homogeneous mixture by gentle shaking and tumbling of the flask.

302.13 Packing Columns

302.13a Aluminum Columns. Plug one end of a straight aluminum column with glass wool. Fill column with a minimum vibration and then tap the plugged end of the floor to settle the packing material. Plug other end of the column with glass wool. Approximately 40 ml (9.5 - 10 g) of the packing material is required to fill the column. After column is filled, form into required shape. An M-shape column is made by making three 180° bends over a 2" cylinder at distances of 18, 36 and 54" from one end of the column. (M-shaped column is used in Micro-Tek Model 2503 R and 2000 R gas chromatographs).

302.13b Glass Columns

- (1) (Burke, J.A., JAOAC, 48, 1037, 1965). Fill column through a funnel attached to one end. In straight or coiled columns, plug the exit with glass wool before adding packing. In straight or "U"-shaped columns, settle packing by tapping the column with a pencil and by gently tapping the column on the floor. Settle packing in coiled columns by attaching a water aspirator to the exit and tapping the column lightly. Fill the injection end loosely with about 3" of glass wool, leaving room for the flash heater and entrance of the needle.
- (2) Fill column through a small funnel with dry, coated material while

holding column in near-horizontal position to minimize breakage of particles as they fall to bottom of tube. Fill the exit arm of U-tube to top, and insert small plug of glass wool. Plug should be a tight ball, but should not be pushed hard against coated material and thus cause localized breakage of particles. Insert one-hole rubber stopper. Fill the inlet arm to about 2" below carrier gas side-arm so that the material will be level with the exit end of tube after packing. Insert a plug of glass wool about 1" long into inlet end. It may be necessary to add more material after preliminary pressure-packing, but do not allow coated material to extend into flash heater area of tube after packing operation is completed. Lay the column on a padded flat surface, applying about 30 psi nitrogen at inlet and vacuum at exit using clamp-on Tygon tubing, and tap column, gently with a heavy rubber stopper on a 1/4" glass rod. Start tapping at exit and work slowly to inlet end. Make one or more cycles of tapping assure that material does not pack appreciably tighter. Slowly release vacuum, reduce pressure, and wait at least one minute after pressure gauge reads zero before removing connecting tubing. Completely fill the upper part of the inlet arm with a tight plug of glass wool, again taking care not to crush particles. The column is now ready for conditioning.

302.14 Conditioning (Burke, J., JAOAC, 48, 1037, (1965); Burke, J.A., and Holswade, W., JAOAC, 49, 374, (1966); Giuffrida, L., and Ives, N.F., private communication, 1965).

Column conditioning (heating) well above operating temperature for considerable time prior to use eliminates substrate "bleed" and improves the chromatography of several compounds. The importance of preventing column bleed cannot be overemphasized. If the liquid phase is bleeding from the column, stable day-after-day performance will be

impaired and frequent detector cleaning will be necessary, sensitivity will change, quantitative results will probably be affected, baselines will drift, and clean-cut chromatograms will not be obtained. Minimum conditioning time to eliminate bleed is over a weekend at 250°C with the carrier gas flow on.

Columns with the combination QF-1/DC 200 substrate should be conditioned at 250°C with nitrogen flow for 3-5 days instead of the 2 days normally required to eliminate all bleed from a DC 200 column. Failure to eliminate all bleed may necessitate electron capture detector cleaning during the first 2-4 weeks of use.

The use of Gas Chrom Q as solid support and siliconized glass wool at the ends of the columns produces best results. Problems in chromatography are minimized with these.

Injection of large quantities of compounds that are difficult to chromatograph may improve their chromatography. However, some pesticide chemicals, such as endrin, Perthane, methoxychlor, Kelthane, tetradifon, chlorobenzilate, Prolan, captan, esters of 2, 4-D, pentachlorophenol, malathion, methyl parathion, Guthion, and Co-Ral, may not chromatograph well until the column is more "thoroughly conditioned." The possibility of adsorption, degradation, or some other effect by the column should not be discounted if the compound shows tailing or little or no response, or if multiple peaks are obtained.

Chromatography of some compounds will not be satisfactory until the column has been used from 2 weeks to a month. This is a distinct disadvantage; it results in lost time and poor analysis. Injection of cleaned up sample extracts appears to improve the column, but no definite procedure has been found to suffice. Conditioning periods of 3-4 weeks at 250°C with a N₂ flow of about 100 ml/min results in improved columns.

302.15 Contamination or Deterioration

302.15a Signs

- (1) Significant decreases in peak height (in particular heptachlor and DDT). This may also be a sign of incomplete column conditioning.
- (2) Loss or rearrangement of DDT peak.
- (3) Loss of resolution.
- (4) Erratic recoveries.
- (5) Nonsymmetrical peaks.

302.15b Causes. Column deterioration is most often caused by lack of adequate cleanup of samples injected onto the column. Extracts of materials containing large amounts of fats or oils, e.g., dairy products, animal tissue, cottonseed meal, fish oils, etc., are particularly difficult to cleanup thoroughly. The oily material that may remain when such an extract is concentrated to a fraction of a milliliter can cause irreversible damage if injected into a GLC column. Waxy or colored coextracted material may also damage the GLC column; however, their effect is not so readily apparent as is that from oily material.

Small amounts of colored matter (presumably carotenes) do not seem to harm the column. However, the injection of any extract that is not well cleaned up should be avoided. The user of GLC must determine if individual samples are suitable for analysis. In some instances, it will be necessary to reject samples or perform further cleanup prior to analysis.

302.2 Standards

302.21 Standard Mixtures. Inject appropriate standard mixtures each day before any sample injections. Repeat the injection as often as necessary but at least twice during an 8-hour period.

302.22 For Quantitation. Inject immediately after sample with suspected significant pesticide residues. Choose injection to yield approximately the same area as the sample peak.

TROUBLE SHOOTING THE GAS CHROMATOGRAPH

I ROUTINE FOR DAILY OPERATION OF GAS CHROMATOGRAPH

- A Turn on all electronics for warm-up.
- B Check system for gas leaks and then adjust gas flow.
- C Check recorder zero and note baseline at various sensitivities.
- D Inject standards and compare with previous runs.

II PROBLEMS AND CAUSES (ELECTRON CAPTURE)

- A Erratic baseline noise. (In excess of 4% of half of maximum sensitivity).
 - 1 Column operating temperature too high.
 - 2 Column substrate coating weight excessive (in excess of 7%).
 - 3 Columns insufficiently conditioned.
 - 4 Contaminated system.
 - 5 Insufficient bakeout time with pure gas flowing.
 - 6 Internal recorder signal connections improper.
 - 7 Signal cable loose.
 - 8 Loose ground.
 - 9 Effluent end of Electron Capture Detector exposed to turbulent air.
 - 10 Intermittent Electron Capture Detector heater.

- 11 Cracked Electron Capture Detector housing.
- 12 Effluent end of detector shorting to case.

B Slow Regular Noise

- 1 Insufficient pressure differential between carrier supply regulator and column head pressure at the maximum analytical column operating temperature.
- 2 Column temperature too high.
- 3 Condensation somewhere between end of column and effluent end of Electron Capture Detector.
- 4 Defective carrier flow regulator.

C Insufficient Sensitivity of Response

- 1 Using metal columns
- 2 Septum leaking
- 3 Bad leak
- 4 Cold spot
- 5 Diluent flow too high or too low.
- 6 Improper carrier gas.
- 7 Carrier gas contaminated with water.
- 8 Carrier gas contaminated with oxygen.
- 9 Operating Electron Capture Detector electrode potential in DC Mode incorrectly set.
- 10 Recorder incorrectly connected to instrument signal output terminals.

- 11 Depleted (weak) source element.
- 12 Pulse rate too fast, in pulse mode operation.
- 13 Pulse generator not operating, in pulse mode operation.
- 14 Cracked Electron Capture Detector housing.
- 15 Leaking Electron Capture Detector housing.

D Baseline Drift Upscale

- 1 Column temperature programming (thermal upscan).

E Baseline Drift Downscale

- 1 Contaminated sample system.
 - a The "proper solvent" is benzene, hexane, or similar non-adsorbing hydrocarbon solvent.
 - b Chlorinated or other electron absorbing solvents should be avoided except as a last resort in cases of extreme contamination.
 - c More generally the following solvents and cleaning method must be avoided:
 - 1) Ethyl or Methyl Alcohol
 - 2) Water
 - 3) Organic Acids, Acetic Acid, etc.
 - 4) Phenolic Solutions
 - 5) Anything containing Labile, Acting Hydrogen.
 - 6) Scrubbing the source by ultra-sonic cleaning.
- 2 Column bleed.

F Excessive Tailing From Sample Solvent Peak

- 1 Premature vaporization of sample at in-side end of septum.
- 2 Solvent
- 3 Diluent gas flow rate insufficient.
- 4 Sample quantity excessive.

III PROBLEMS AND CAUSES (MICROCOULOMETRIC)

A Positive High Baseline

- 1 Titratable material entering cell.

B Negative Baseline

- 1 Silver from silver acetate electrode entering cell.

C Noisy Baseline

- 1 Stirring bar

D Baseline goes negative at irregular intervals and is slow returning

- 1 Excess silver in cell

E Erratic baseline

- 1 Temperature of cell varying.

F Peaks Overshoot

- 1 Under damping.

G Peaks overshoot but system acts over damped.

- 1 Dirty cell

H Peaks tail

- 1 Over damped

Trouble Shooting the Gas Chromatograph

I Odd Peaks

- 1 Contaminated system

J Peaks Break-up

- 1 Contaminated system

K Low Recoveries

- 1 Leaking system
- 2 Improper combustion

This outline was prepared by J. I. Teasley,
former Supervisory Chemist, Southeast
Water Laboratory, EPA, Athens, GA.

Descriptors: Chemical Analysis, Chroma-
tography, Instrumentation, Water Analysis

SAMPLING FOR PESTICIDE RESIDUE ANALYSIS

I REQUIREMENTS FOR SATISFACTORY SAMPLING

- A The sample must be valid. Validity is achieved by selecting the sample in a manner which assures that each unit of material in the batch being sampled has an equal chance of being chosen for the extraction and final test. This process is known as randomization, and a sample taken in this manner is considered a random sample.
- B The sample must be representative. A representative sample is not only a random sample but the proportion of each type of plant material, for example, in the sample is identical with that of the gross sample from which it was selected.

II VARIABLES TO BE CONSIDERED

In establishing that the sample meets the above requirements, the following variables must be considered:

- A Source of the sample - from a 100-acre truck farm or a vegetable garden, from a bushel basket or a carload.
- B Size of the raw commodity - a mustard seed or a watermelon.
- C The nature and stability of the raw commodity - raw milk or canned beans.
- D Method of applying the pesticide - by hand or aerial spraying.
- E Intended use of the crop - is the whole crop consumed or only part?
- F The on-crop behavior of the chemical - surface coating or systemic.
- G Possible skips in the application of the insecticide, and drift from adjacent plots.
- H Variations in the size and/or density of the crop.

III COLLECTION OF THE GROSS SAMPLE

- A The gross sample size can be estimated by a trial run from which the magnitude of the population mean and variance is obtained.
- B A decision is then made on the accuracy desired and the sample size may then be estimated from the following formula:

$$n = \left(\frac{st}{h} \right)^2$$

where

n = sample size

s = standard deviation

h = desired accuracy expressed as a percentage of the mean

t = student's from statistical tables

- C While generally a representative and valid sample can best be collected by random sampling, occasionally, in order to take into account all variables, samples are collected systematically or at predetermined stations.
- D Several gross samples may be collected from the same plot in order to be assured of the accuracy of the data. For practical or economical reasons this "replication" may not always be possible.

IV CLASSIFICATION OF RAW AGRICULTURAL COMMODITIES

- A In order to simplify consideration of suggested field sampling procedures, it is helpful to classify the various raw agricultural commodities into the following general or typical categories:
 - 1 Large tree fruits (such as apples, avocados, citrus and peaches).
 - 2 Nut crops (such as almonds, chestnuts, pecans and walnuts).

Sampling for Pesticide Residue Analysis

- 3 Small fruits (such as blueberries, currants, grapes and cherries).
- 4 Legumes for food (such as beans, lima beans, peas and soy beans).
- 5 Legumes for forage (such as alfalfa, clover and vetch).
- 6 Grains for food (such as barley, corn, oats and wheat).
- 7 Grain for forage (such as corn and sorghum forage).
- 8 Leafy vegetables (such as collards, kale and leaf lettuce).
- 9 Head crops (such as cabbage, cauliflower, head lettuce and celery).
- 10 Cucurbits or vine crops (such as cantaloupes, squash, and watermelons).
- 11 Perennial crops (such as asparagus, artichokes and rhubarb).
- 12 Solanaceous fruits (such as eggplant, peppers and tomatoes).
- 13 Root crops (such as beets, carrots, onions, potatoes and radishes).

V FIELD SAMPLING

- A Large tree fruits - there are a number of factors that influence the residue level at harvest time.

- 1 Variations in spray penetration through the foliage.
- 2 Differences in exposure of various portions of the tree to weather.
- 3 Differences in the chemical and physical nature of the surfaces of the crop.
- 4 Because of these factors a gross sample of about 100 lbs is taken.

- a If the plot is small, it is necessary to sample each tree.
- b If the plot is large, samples are taken at random from no fewer than 16 trees.
- c In all cases, fruit is taken from all four quadrants of each tree sampled at both inner and outer levels, selecting the fruit in proportion to its abundance.
- d The gross sample is reduced by careful mixing and quartering to approximately 25 lbs.(Figure 1)
- e The composite sample is placed in a kraft-paper or heavy plastic bag or the equivalent pending further reduction in sample size and subsampling.

- 5 Subsampling is accomplished by cutting each fruit into halves, mixing and quartering the pieces until a 6-lb sample is obtained. If feasible, the 6-lb sample is divided into 3 equal representative portions. The resulting subsamples are placed in suitable containers labeled and frozen.

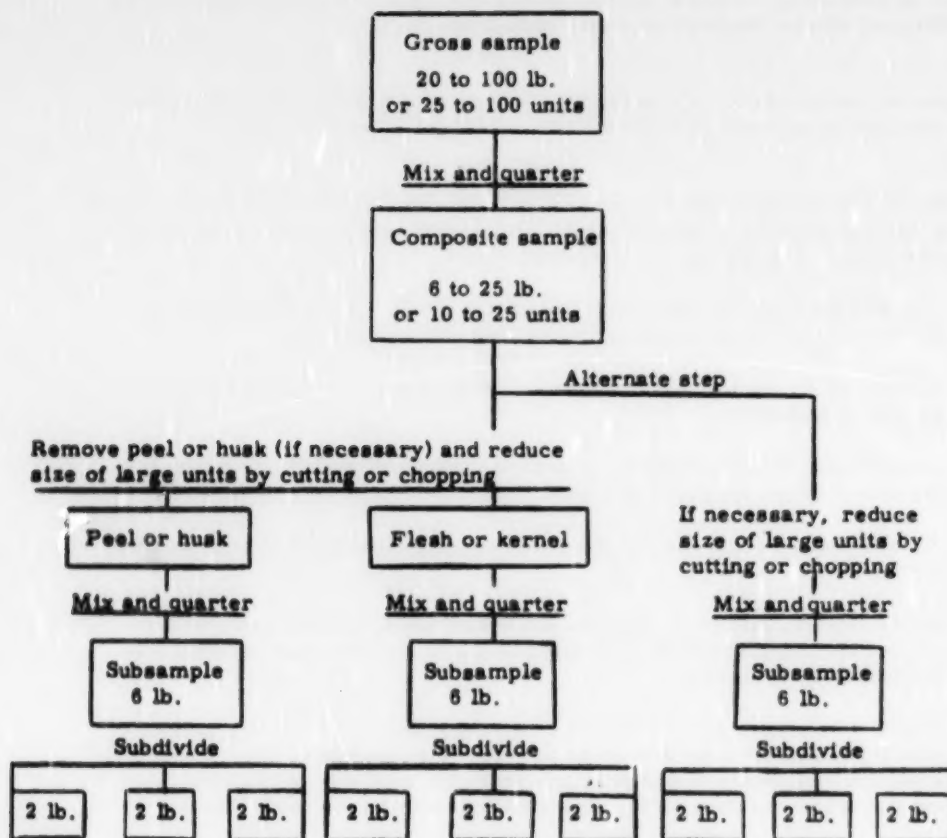
- a Where the whole fruit is to be consumed in the fresh state, the fruits or segments are analyzed with the peel intact. (Table 1)

- b If the fruit is to be eaten after peeling for canning or freezing, the fruit should be peeled and the subsample taken from the peeled material.

- B Nut crops - most procedures applicable to tree fruits are applicable to nut crops.

- 1 Since only the nut meats are consumed, the hulls and shells are removed and discarded.

Figure 1
TYPICAL STEPS IN REDUCTION OF GROSS SAMPLE



From: Gunther, F.A., Residue Reviews, Volume 3. Academic Press, 1963

Table 1. PREPARATION OF RAW AGRICULTURAL COMMODITIES

Remove only obviously decomposed leaves, berries, etc. Do not wash*, dust, cull, strip, or otherwise use procedures which might be used in preparing the food for consumption. Remember that tolerances have been established on the commodity as shipped unless otherwise specified.

*Samples of root crops to be analyzed for residues of pesticides applied prior to harvest should be freed of adhering soil by a light rinse in water.

See Pesticide Regulation 120.1 (j) for specific exemptions from the requirement that the raw agricultural commodity to be examined shall consist of the whole raw commodity. Exemptions as of this date are:

1. The raw agricultural commodity bananas, when examined for pesticide residues, shall not include any crown tissue or stalk.
2. Shell shall be removed and discarded from nuts before examination for pesticide residues.
3. Caps (hulls) shall be removed and discarded from strawberries before examination for pesticide residues.
4. Stems shall be removed and discarded from melons before examination for pesticide residues.
5. Roots, stems, and outer sheaths (or husks) shall be removed and discarded from garlic bulbs, and only the garlic cloves shall be examined for pesticide residues.
6. Where a tolerance is established on a root vegetable including tops or with tops, and the tops and roots are marketed together, they shall be analyzed separately and neither the pesticide residues on the roots nor the pesticide residues on the tops shall exceed the tolerance level.
7. The crowns (leaves at the top of the fruit) shall be removed and discarded from pineapple before examination for pesticide residues.

From: FDA, Pesticide Analytical Manual.

2 If the outer edible hulls are to be used for animal feed, it is desirable to analyze them.

3 Generally, it is not necessary to keep the samples or subsamples frozen.

C Small fruits (Blueberries, currants, grapes and cherries).

1 It is advisable for two persons to pick from opposite sides of the row, simultaneously.

2 Sufficient fruits are taken from 12 to 24 sampling stations to provide a gross sample of 20 lbs.

3 The gross sample is reduced immediately as before to a 6-lb sample, which is subdivided into 3 equal subsamples, placed in a suitable container and frozen.

D Legumes for food (peas, lima beans).

1 Sample is gathered as was done for small fruit, the gross sample in case of peas is hulled. The gross sample is then subdivided as before and frozen.

E Legumes for forage (alfalfa, clover, hay).

1 Total harvest is practiced in the sample selection area.

2 Twenty-four random sampling stations are established. The forage is cut to the normal harvest height and a 1-lb sample is reaped from each station.

3 The 1-lb samples are cut into one-inch lengths, combined, mixed, and reduced by quartering to a 6-lb sample.

F Grains for food (barley, oats, wheat).

1 Since mechanical harvesting is generally practiced, the 24 one-pound samples needed are collected from the discharge chute of the combine, uniformly spacing them over the entire plot.

2 The resulting gross samples are combined and quartered as before.

3 Corn plots can be adequately sampled by collecting five or six mature dry ears from each of 24 sampling stations in the field or plot. The husks are stripped off and the kernels removed from the cob.

G Grain forage (corn or sorghum).

Sample as for legumes. If green or wet, the final samples are frozen.

H Leafy vegetables (collards, kale, and leaf lettuce).

Depending on the size of the plot, 12 to 24 sampling stations are planned.

1 Only the forage normally used for food is included. The samples are composited and reduced by mixing and quartering.

2 Since leafy crops are always washed before eating, the components in the 6-lb sample are washed, drained, chopped into small pieces and frozen.

I Head crops (cabbage, head lettuce, celery).

Twenty-five heads from each plot are collected. They are trimmed to market condition.

1 The heads are quartered and alternate quarters are combined to produce the gross sample.

2 The resulting sample is reduced by cutting each quarter into one-inch size pieces, mixing, quartering and freezing.

J Cucurbits or vine crops (melons and squash).

The gross sample is collected in a manner similar to that for head crops.

- 1 Depending on the use or how normally eaten, the rind may or may not be removed. If not removed, the crop is washed and then quartered as was done for head crops.

K Perennial crops (asparagus, artichokes and rhubarb).

Component units are collected from 12 to 24 stations. Each component unit is trimmed, washed as for market, and chopped into small pieces. This gross sample is then well mixed and reduced by quartering.

L Solanaceous fruits (eggplant, peppers and tomatoes).

The procedure is similar to that for head crops.

M Root crops (beets, carrots, onions, potatoes and radishes).

Component units are collected as before from 12 to 24 stations to make a gross sample of about 50 lbs (20 lbs for small roots). The gross sample is reduced to one-half by quartering after removing tops and roots.

- 1 The sample is then washed free of dirt, chopped into one-inch pieces, mixed, quartered, and the final 6-lb sample divided into three portions which are frozen.

VI SAMPLING DURING PROCESSING

A Obtain sample at random at point just prior to the final packaging.

- 1 Select three, 4-lb samples from the first, middle and last of the material being processed.
- 2 Carefully mix, and quarter to reduce the sample to three 2-lb subsamples and freeze.

VII SAMPLING OF PRODUCE AT THE MARKET (Table 2)

A Thirty years experience in pesticide residue enforcement work in California indicates that the size of samples shown (Zweig, Vol. I, page 607) for typical commodities have been suitable. In the regular daily survey of produce on the market, a relatively small "routine sample" is drawn for analysis. If this is found to contain over-tolerance residue, the inspector returns, quarantines the lot, and draws a larger "confirmation sample" to check the findings for possible prosecution. In some cases, where a lot is under suspicion for some reason, the larger lot is drawn at the start.

B The amount of fruit, vegetable, hay or other commodity drawn for an official sample must be a compromise between the level of representativeness desired and the costs involved. Analyzing one apple out of a carload does not seem adequate, and analyzing an entire boxful out of a carload seems unnecessary. (Table 3)

VIII BULK AGRICULTURAL MATERIALS (grains, flour, fertilizers, nuts, dried milk, beans, seeds)

A Sampling of bins or elevators are best sampled when being loaded or unloaded. Samples should be collected by momentary interruption of the flow into or out of the bin, at regular intervals during entire filling or emptying period.

B Small bins, trucks or freight cars are best sampled with a grain trier or thief. A composite sample of about 5 kg. in weight is collected from individual probings.

C Bags and barrels can be sampled with a smaller thief. The trier should be introduced to pass diagonally through the sack or barrel from one corner of the top to the opposite corner at the bottom.

Table 2. SIZE OF SAMPLES FOR DETERMINING PESTICIDE RESIDUE

Commodity	Routine sample	Confirmation sample
Apples or pears	6 to 10 fruits	12 to 18 fruits from several containers
Artichokes	2 to 4 heads	4 heads from several containers
Beans, lima or snap	About 2 lb	2 to 3 lb from several containers
Berries	Collect individual berries at random to represent a cross-section of the lot, such as top and bottom fruit in individual baskets and from several trays throughout the lot. Draw at least two baskets, as sold.	About a pound or more scattered fruit or whole baskets from several trays
Broccoli	About 2 lb or a bundle, if tied	About 3 to 4 lb or 2 bundles, if tied, from different containers
Brussels sprouts	1 to 2 lb	About 2 lb from several containers
Cabbage	2 heads from different containers	3 heads from different containers
Cauliflower	1 head	2 to 3 heads from different containers
Celery	1 or 2 stalks, 3 stalks, if hearts	3 stalks from different containers
Corn, sweet	4 ears from different containers	4 ears from different containers
Cucurbits	About 2 lb	About 3 lb from different containers
Grapes	About 2 lb broken clusters from scattered bunches	2 to 4 lb in whole bunches or scattered clusters from several containers
Greens	2 bundles from different containers	4 bundles from several containers
Hay, fodder	About 2 lb	About 2 lb
Lettuce	2 or 3 heads from different containers	3 or 4 heads from several containers
Peas	1 to 2 lb	2 to 3 lb from several containers
Peppers	About 2 lb	3 to 4 lb from several containers
Tomatoes	6 to 10 fruits	12 to 18 fruits from several containers

From: Zweig G., Analytical Methods for Plant Growth Regulators and Food Additives, Volume I, Principals, Methods and General Applications. Academic Press, 1963.

Table 3. SAMPLING SCHEDULE FOR PESTICIDE RESIDUES

This schedule assumes that analysis will be made on a composite of portions from all of the subsamples. The composite often is larger than needed for analysis because of the necessity of obtaining a sample representative of the lot. Each subsample must also be large enough and taken in such a manner that it represents the shipping container. Collect only one subsample from the shipping unit and pack separately.

Treat each identifiable grower's mark or lot number in a shipment as a separate sample except for comingled lots containing many growers. In random sampling, be careful to collect not more than the proportional amount from facing layers. When sampling from loading or unloading carloads, select subsamples at intervals so as to obtain a sample representative of the carload.

SAMPLE SCHEDULE

Collect 2 pound subsamples in accordance with the following table:

Number of Subs	
<u>Lot Size</u>	<u>No. of Subs</u>
Up to 20	4
20 to 50	6
50 to 100	8
100 to 200	10
200 to 300	15
above 300	20

Bulk shipments of Hay, Corn and Silage; Collect subsample from each 5 ton unit with a maximum of 20 subs.

SAMPLE HANDLING

Hold samples in cold storage until ready to be shipped or delivered to the laboratory only if normally held or shipped under refrigeration in commercial practice.

From: FDA Manual

IX MEAT AND MEAT PRODUCTS

- A A systematic sampling of meat, would appear to be ideally carried out at slaughter houses. It may be possible to infer the insecticide content in the muscle from measurements of blood or milk. It is known, of course, that the fat contains concentrates of most chlorinated-hydrocarbons.
- B If meat samples are required, it is probably considerably more practical and economical to sample prepared cuts from packaging plants or shops. Poultry supplies may be obtained in a similar way.
- C To draw representative aliquots of soft tissues it is necessary to pass the tissue through a meat grinder about three times. These samples should then be mixed by hand, quartered, and three 2-lb samples reserved for analysis.

X FISH AND OTHER MARINE PRODUCTS

- A It may be most convenient to sample after the fish have been landed and sorted out. A multi-stage sampling scheme is likely to be necessary, taking into account the number of ships, type of fish, etc.
- B The preparation of the fish should approximate the preparation given the fish prior to consumption. For small (up to 15 in.), after cleaning, a longitudinal half of the fish forms a suitable portion to be analyzed. This should be passed through a food chopper three times and thoroughly mixed each time.
- C Large fish can be sampled by cutting three transverse slices, one inch thick; one from immediately behind the pectoral fin, one halfway between the pectoral fin and vent, and one immediately behind the vent. The slices are then treated as for smaller fish.
- D Shellfish will have to be removed from the shell or otherwise prepared as is customary and then handled as described for fish.

XI MILK SAMPLING - Dairy farms, processing plants and stores are the most probable locations for collecting milk samples.

- A Since a few pasteurizing plants usually supply the major portion of the milk, sampling at the plant decreases the task in sample collection.
- B Random collection of milk samples from the conveyor belt, at selected intervals (during a four-hour run - one quart per hour).
- C Samples are combined and subsamples taken for analysis. Samples should be kept refrigerated until analyzed.
- D In some dairies, milk is pumped from tank cars and trucks through pipes that have built-in sampling devices. Drip sampling from such a device will provide an ideal method of sampling the throughput of milk over a period of one or more days.
- E Samples may also be taken by dipper from several locations through the tank. If cream has started to separate, then the use of a "thief" is indicated or agitation of the tank before sampling.

XII SOIL SAMPLING - Since all values are to be referred to the surface area, it is necessary to know the exact diameter of the core or boring produced.

- A If the soil has a grass or crop cover, the sword should be cut and removed from a number of plots of one square meter.
- B Corings should be taken from the center of each plot to a depth of at least 4 inches. A 4-inch "bogey" hole cutter as used in golf courses is a very suitable tool.
- C Ten to twenty cores representing a total surface area of at least 200 square centimeters are usually recommended.

- D The first 2 inches of the core representing the grass or crop cover and roots should be separated from the underlying soil.
- E Once the samples have been collected, the corings representing each layer of soil should be combined, quartered and divided into three 2-lb samples for analysis.

XIII WATER SAMPLING

- A Grab sampling - merely means an instantaneous sample of water is taken at a given station.
 - 1 Grab sampling is usually sufficient for lakes and reservoirs which are not subject to rapid (hourly) transitional changes.
 - 2 Grab samples are best obtained by direct filling of the sample container.
 - 3 To provide a more representative sample, samples should be taken at various depths and locations.
- B Continuous and automatic samples are more appropriate for sampling rivers and streams.
 - 1 Samplers have been designed to collect water samples at a rate proportional to either stream flow or time.
 - 2 In general, locations should be near the mouths of rivers so as to cover the major river drainages in the United States.
- C The sampling of finished or potable waters is best accomplished at the treatment plant. Carbon filters may be useful.

XIV AIR SAMPLING

- A It has been demonstrated that Greenburg-Smith impingers containing ethylene glycol are efficient traps for organo-phosphorous and halogenated hydrocarbon insecticides both in the vapor phase and as dusts. The air is drawn through the impingers by means of a vacuum pump.
- B The components of the sampling system have been housed in a convenience box for field sampling and has been equipped with timers to permit intermittent samples to be taken over long periods of time. The instrument may be preset to sample from 1 to 60 minutes out of each hour or from 1 to 10 minutes out of each 10-minute period.
- C An elapsed time meter records the total sampling time which can be converted into volume of air since the air flow is a constant 10.3 liters per minute.

XV STORAGE OF SAMPLE - When the crop is received from the field, it must be properly coded and recorded.

- A If it is a raw commodity, it should be stored in a deep-freeze until it can be further processed. Samples should be kept in polyethylene bags and each sample should contain an identification sheet.
- B The analyst should also determine whether the particular chemical to be analyzed decomposes, volatilizes or undergoes chemical change during storage.

XVI PREPARATION OF SAMPLE FOR ANALYSIS (Table 4)

Table 4. GUIDELINES FOR COMPOSITING

<u>Animal Tissue</u>		Grind Ca half of each sub (meat grinder); composite 100 g from each sub and grind again.
<u>Dairy Products</u>		Equal weight from each sub. Grind or dice or blend.
<u>Eggs</u>		Equal number of units from each sub, for total of 6-12. Blend.
<u>Feed, Animal</u>		200 g from each sub (quarter subs down to 200 g where necessary); wet feeds (silage) 100 g from each sub.
<u>Forage</u>		Quarter each sub down to 200 g; composite 200 g from each sub. Chop fine. Where necessary, grind in Wiley Mill without screen; then with screen in.
<u>Fruits</u>	<u>Large</u>	(Apples, pears, tomatoes, etc.) Equal number of units from each sub. Chop or blend.
	<u>Small</u>	200 g from each sub. Chop or blend.
<u>Grains</u>		100 g from each sub. Grind in Wiley Mill or equivalent.
<u>Hay</u>		200 g from each sub. Chop or grind.
<u>Milk</u>		100 g (ml) from each sub after thorough shaking.
<u>Nuts</u>		Remove shells. Composite equal number of units (equal weight) from each sub. Chop or grind.
<u>Oils</u>		Equal weight or volume from each sub.
<u>Seeds</u>		100 g from each sub. Grind.
<u>Spices</u>		200 g from each sub. Grind or chop.
<u>Vegetables</u>	<u>Head</u>	Quarter each head in the sub. Take two opposite quarters from each head and chop into one to two-inch pieces with a knife. Mix well. Composite 200 g of chopped product from each sub and chop entire composite in a food chopper.
	<u>Leafy</u>	<u>Leaf Cut</u> - Mix sub well and select leaves at random until a 200 g portion is obtained. Composite 200 g from each sub and chop the entire composite in a food chopper. <u>Field Cut</u> - (Leaves attached to stalk). Select bunches at random until a 500 g portion is obtained. Composite 500 g from each sub and chop entire composite in a food chopper.
	<u>Pod</u>	(Beans, peas, etc., also asparagus) 200 g from each sub.
	<u>Root</u>	Equal number of units from each sub.
	<u>Stalk</u>	(Celery, broccoli, etc.). Quarter each sub lengthwise and proceed as in "Head Vegetables."

From: FDA, Pesticide Analytical Manual

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DESIGN OF A PESTICIDE SAMPLING PROGRAM FOR THE WATER ENVIRONMENT

I The first step in the design of a sampling program is to specifically and carefully define the objectives of the study.

A Proper design of the sampling program (and adherence to it) will be, given an adequately evaluated analytical method, the most critical factor to determine if the objectives of a study are met.

B Ideally, the sampling program should be statistically designed in all respects so that confidence limits can be stated for every significant conclusion. Realistically, this standard is often lowered to some less precise, less demanding level due to practical reasons such as limitations of manpower, money and time. The researcher then has the responsibility to limit the precision and accuracy of the conclusions he draws to that of the sampling program and analytical methods employed.

1 Statistically, precision refers to the reproducibility among replicate observations (whether samples or analyses).

2 Accuracy refers to degree of difference between observed and known or actual values. Inaccuracy does not destroy the value of data if the degree and precision of the error is known and taken into account.

II The second step in sample program design is to select the parameters of direct or indirect importance to be measured.

If the objectives have been carefully defined, the kind, accuracy and precision of the information required will usually be obvious. Frequently, limitation of technology and resources available to do the study will in turn limit the range of the objective.

A Qualitative Data

Qualitative information is the easiest to obtain because it only involves accuracy of identification, not numerical precision and accuracy.

Almost invariably the next question following a negative qualitative finding will be, "What was the lowest level of detectability of the analytical method used?"; or, following a positive qualitative finding, "How much?"

B Quantitative Data

Quantitative answers as to "how much" was in the sample require knowledge of the sum total accuracy (efficiency) and precision of the analytical technique.

Extrapolation of the data obtained from the sample(s) to an accurate statement of "how much" was in the stream (water, mud or organisms) demands that we also know how accurate and precise was the sampling program.

Good statistical design of the sampling program and analyses of the resulting data may produce a statement of precision, but tell nothing about the accuracy of the sampling method employed. The sampling method must itself be evaluated either by comparison with another method of known accuracy or by controlled condition tests.

The fact that a chemical has been found to be toxic to a pest, not similarity of chemical nature, places it in the "Pesticide" category. Therefore, the precision and accuracy of the sample program design, sampling method, and analytical technique must be determined for each and every pesticide.

The present state of this relatively new art is far short of that evaluation goal. As a result, "pesticide" literature is replete with qualified conclusions such as, "no less than (x) amount of (y) pesticide was present," and, "at least (x) amount was present." In many additional instances, qualified conclusions would have been more valid than the definitive statements issued.

III Frequency of sampling is often of critical importance. Again the objectives of the study will determine this, but knowledge of the time behavior of the pesticide, the environment and the source of the pesticide is required.

IV LOCATE SAMPLING STATIONS

The location of the sampling station must be representative of the water body being sampled. The greater the variability of the water mass, the more samples must be taken.

A Hydrologic Considerations

The assumption that water is homogeneous is tempting but often not true with respect to a number of parameters. For example:

The velocity of flow in a straight stream channel is greater near the surface at mid-channel than at either side or at the bottom.

Sediment particles are heavier than water, and this fact, combined with the distribution of velocity described above results in a sediment concentration gradient represented in Fig. 1b.

The sediment discharge (weight/unit time/unit area) in this model is the product of the velocities (volume/unit time/unit cross sectional area) X sediment concentration (weight/unit volume), Fig. 1c.

The vertical distribution of silts (diameter 0.05 to 0.002 mm) and clays (diameter < 0.002 mm) concentration tends to be fairly uniform from surface to bottom (Fig. 1b). The approximate sixfold increase in sediment concentration from surface to bottom

is due to sands (diameter 2.00 - 0.05 mm). The average surface area per unit weight of the silt-clay fraction is roughly 1000 times that of the sand fraction. Therefore, the six-fold increase in sediment concentration toward the bottom increases the particle surface area the equivalent of from 1000 to only 1006.

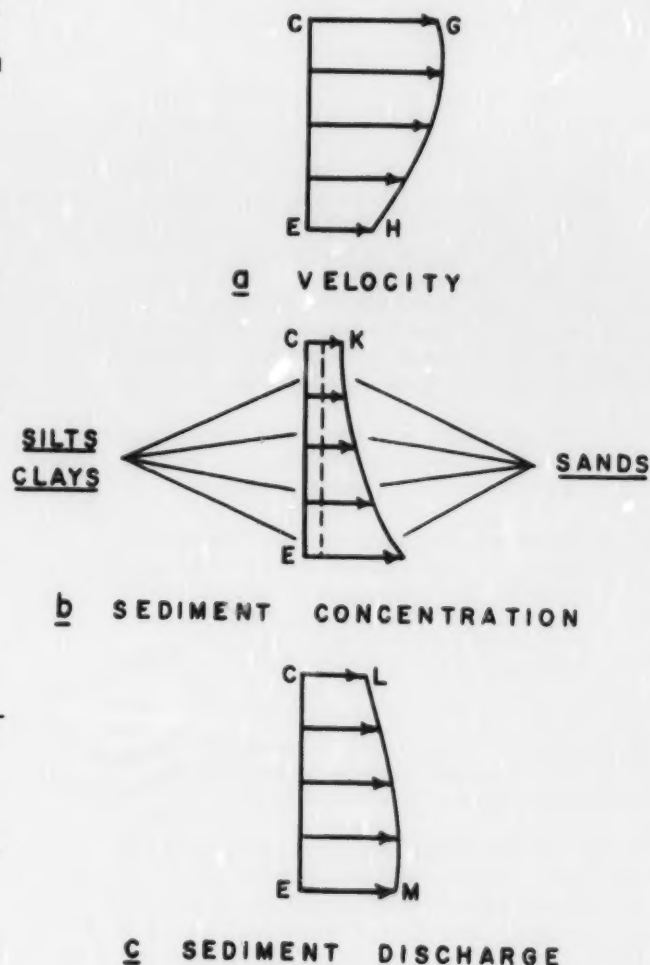


FIG. 1-- STREAM DISCHARGE

Since some pesticides, most notably chlorinated hydrocarbon insecticides and the bipyridylum group of herbicides have a strong affinity for sediment particles, the amount, distribution and discharge of particle surface area available for adsorption in a stream may be an important sampling problem.

We see from the above discussion that a sample from any depth in the vertical water column possibly would yield data representative of the instantaneous concentration in the entire column. However, if an accurate measure of total discharge of a pesticide is desired, variation in velocity of flow will have to be considered.

Few streams remain straight, even channels for any great distance. The flow pattern is considerably complicated by a bend in the stream and the extent of this is dependent on the angle of bend. One over simplified phenomena that takes place is indicated in Figure 2. The water twists around like the braids of a rope and the implications in this instance are evident ... but not to the sampler.

Presence of pool and riffle areas, common to most streams, further complicated the picture for the sampler.

More energy is required to produce turbulence than laminar flow. Hence, water

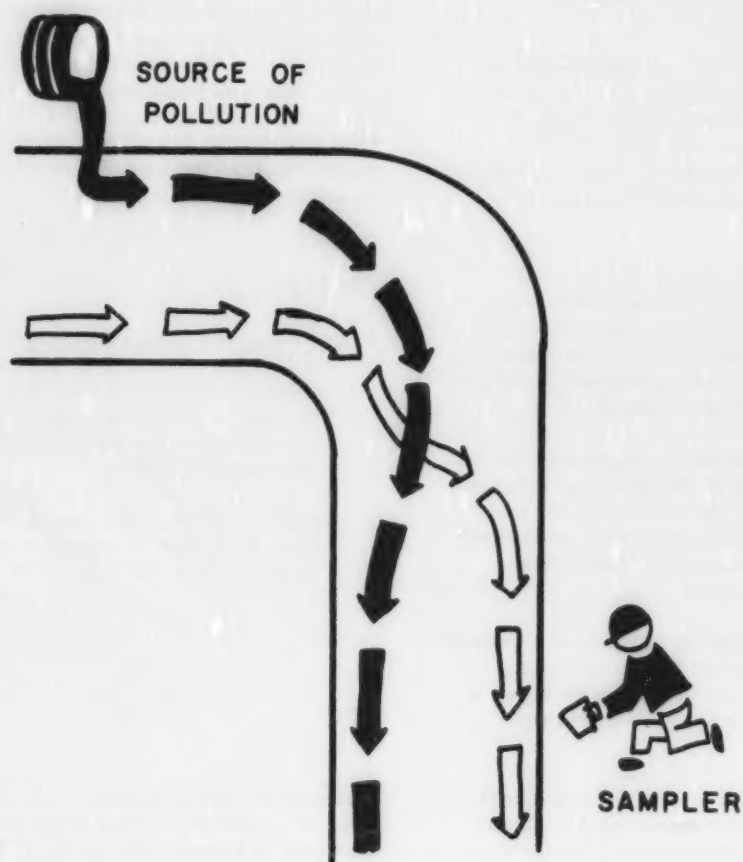


Figure 2

is more easily moved enmasse than mixed. Increased density caused by lower temperature, and higher concentrations of dissolved solids or suspended sediment enhance this tendency not to mix. It sometimes happens that the more dense water slides under, the less dense, and the two masses flow along in layers for a considerable distance before mixing.

Water from tributaries frequently flows alongside the receiving stream's water. This is exemplified by the Mississippi River downstream of its confluence with the Missouri. The water masses of the two rivers frequently maintain photographically distinct identities for a considerable distance downstream ... the muddy brown water of the Missouri staying to the State of Missouri side.

Impounded waters offer an additional set of potentially complicating factors to the sampler. Most of these arise from the fact that there is less mixing and more likelihood of stratification due to establishment of temperature and/or chemical density gradients.

- B Since water is the transporter of sediments, the very brief consideration of hydrologic variables above should amply demonstrate that the sampler must also expect variability in the composition and distribution of bottom sediment.
- C The distribution of living organisms in the water environment is usually highly variable and clumped, the net result of the complex, interacting, dynamic, processes of its living and non-living (especially hydrologic) components.

Non-motile planktonic organisms depend on water movement for suspension. Their distribution in the water mass is determined primarily by hydrologic factors.

Motile organisms gather in places offering optimum environmental conditions such as light, temperature, oxygen, nutrients or food, velocity of flow, etc.,

and therefore are apt to be more clumped and variable in qualitative and quantitative distribution.

V SELECTION OF SAMPLING METHODS

The selection of the method(s) of sample collection for pesticide analyses is almost entirely determined by the objectives of the study. Implicit is the need to adapt use of presently available sampling methods, or develop new methods to meet the particular demands imposed by the nature and behavior of both the water environment and the pesticide(s) to be sampled and, to select methods which will also provide sufficient residue for the analytical analyses.

Instrumental analytical techniques now employed permit more definitive analyses and detection of lower (parts per trillion) concentrations with extracts from one liter of water than was possible as recently as the late 1950's with extract from 5000 gallons. This advance in analytical methodology has made possible the use of a much broader range of sampling methods.

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COLLECTION AND PRESERVATION OF WATER, MUD, FISH, BLOOD, AND OTHER BIOLOGICAL SPECIMENS FOR PESTICIDE ANALYSIS

I INTRODUCTION

Samples are worthless unless adequately labelled. Information made available should include: date, name of stream or lake, location of sample station, name of collector, type of sample (qualitative or quantitative), volume of water represented or weight where applicable, and a notation as to the types of analyses desired.

The type of label used must be durable and attached to the sample so that it will not come off.

The method of collection will usually be determined by the objectives of the study. The need to adapt and improvise on available methods is the rule rather than the exception.

II COLLECTION OF WATER SAMPLES

A Discrete Grab Samples

The usual technique is to submerge the sample container a few inches below the water surface during filling. This avoids skimming off any floating film which would be least representative of the vertical water column. Discrete samples from various depths can be obtained with a standard Kemmerer sampler.

Grab samples yield Qualitative and Quantitative data but the amount of qualitative "detective" work by corroborative analytical techniques that can be done is limited by the small amount of extract.

The assumption is that the sample grabbed is at least representative of the immediate water mass from which it was taken and, hopefully, somewhat representative of the water that will pass that sampling point during some limited interval of time.

The grab sample is amenable to use in both random and non-random sampling program. The number, frequency, and distribution of samples collected will depend on the study objectives and the variability within the "population" being sampled.

Two types of containers commonly used are one-gallon tin cans and quart-jars encased in individual styrofoam shipping containers. The tin cans offer the advantages of economy (30¢ each) which makes them disposable and eliminates dish washing, and they require no crating or packing for parcel post handling. Disadvantages are that on rare occasions the cans are split by rough handling and they will rust through, so have limited storage time.

All types of containers need to be evaluated for container effect on the pesticide(s) being sampled for. Effect of storage time should also be determined.

Preservation methods will depend in part on the above two effects, on the effect of the preservative itself and on the inherent stability of the pesticide. Biochemical degradation of pesticides in the samples can be controlled by a variety of sterilants including chloroform and mercuric chloride. Postal authorities will sometimes object to handling samples containing poisonous or volatile preservatives.

Some pesticides are so unstable that samples must be extracted immediately in the field with such as ion exchange resins in order to preserve the integrity of the sample.

B Compositing, Proportionalized Grab Sample Method

Equipment is now available for collecting samples from gauged and instrumented streams that are proportional to the flow of the stream. This method is particularly useful, in fact required, to determine the total discharge load of a pesticide from a stream.

A drawback is that the sample intake hose must be located upstream from any influence of the stilling basin at the weir, especially when sediment transported pesticides are concerned. The assumption then has to be that the fixed intake opening will be sampling representatively at all stream gauge heights.

The sample volume varies with stream flow. It is usually too large a volume for shake-out extraction so sub-sampling of the sample is required.

If compositing of the sample is extended to a period of days, the problem of preservation is multiplied.

Many pesticides have a strong affinity for surfaces (such as rubber and plastics) of commonly used collection apparatus. To protect the integrity of the sample, as inert a material as possible (preferably stainless steel) should be used.

C Carbon Adsorption

The technique involves passage of a continuous, constantly controlled volume of water through a column of activated carbon.

The major advantages of this method are that it takes a continuous sample and that it yields sufficient quantities of extract for corroborative, qualitative, analytical analyses.

The precision of the method appears to be satisfactory but the quantitative efficiency is open to many questions. Efficiency of adsorption has already been found to vary dramatically, depending on the rate of

flow through the column and the total volume passed. A broad spectrum of organics are adsorbed by the carbon but it has been estimated that perhaps 95% of the total organic load passes through. Many pesticides are adsorbed by activated carbon but little is known at present about the efficiency of adsorption for specific pesticides. Quantitative statements of pesticide concentration based on carbon adsorption should be restricted to the, "it is certain that no less than (X) amount was present," variety.

III COLLECTION OF MUD SAMPLES

Bottom sediment varies with respect to both particle size composition (absorptive surface) and organic content. Therefore, sample sites should preferably be selected by a random method. As a minimum, a vigorous effort should be made to collect samples representative of the range of variation. About a quart of mud is desirable, whether the sample is a composite of sub-samples or an individual sample. This will provide enough material for several extractions. Actual collection of the mud is accomplished with a variety of samplers, including among others, core samplers, the Ekman dredge and the Peterson dredge.

Mud samples may be preserved with formalin or a variety of other sterilants so long as they do not affect the integrity of the sample.

Some chlorinated hydrocarbon insecticides have been found to maintain concentration for several months when air dried, ground and stored. However, more volatile compounds such as 2-4-D would be preserved longer if the samples were frozen. No sample should be stored longer than absolutely necessary.

IV COLLECTION OF BIOLOGICAL SAMPLES

The general prerequisite for samples to be analyzed for pesticides is that there be sufficient mass. This is a problem especially where planktonic and bottom fauna organisms are concerned. The absolute minimum weight

of sample that can be successfully analyzed is to a great extent dependent on the concentration of pesticide in the sample. As little as one gram may sometimes be sufficient if the analyst knows approximately what concentration to expect. A good rule of thumb is a minimum of ten grams.

Only those collection methods normally employed for ecological studies that are amenable to concentration of mass are useful.

Organisms may be preserved for pesticide analyses by freezing as soon as possible after collection if none other than broad taxonomic identification is desired. It is usually more convenient to preserve samples in the field with either 5 to 10% formalin or 70% ethanol.

Water from which the organisms were collected should not be used to make up these mixtures. Use distilled water. This will eliminate the possibility of pesticides in the water concentrating on the organisms over a period of time.

The preservative (especially ethanol) in which the samples have been stored should be extracted and analyzed for pesticides at least enough times to be sure that exchange of pesticides from the organisms to the preservative is not occurring. This happens frequently, and when it does, the quantity of pesticide in the preservative must be computed and added to the total obtained from the organisms before final computation of concentrations.

A Plankton

The only practical methods of collecting sufficient mass of plankton organisms for pesticide analyses are the use of a tow net behind a boat, or pumping water through a net.

B Benthic or Bottom Fauna

Standard techniques utilizing dredges (Ekman - soft mud, Peterson - firm bottom) in pool areas, Surber sampler or similar device in riffle areas and dip nets in areas thick with vegetation are suitable.

The samples are washed through a hand screen (usually 30 mesh/inch) to remove the mud. Large stones and sticks are removed by hand. What remains is a mixture of organisms and debris. The organisms are then hand picked from the debris. Anyone who has ever picked bottom samples will know that picking 10 grams, including all organisms in the samples, is no small task. If 10 grams of each taxonomic category is desired, the task becomes prodigious.

C Fish

Techniques utilizing seines, gill nets, traps, otter trawls, electric seines, poisoning (rotenone), and angling are suitable. Many of these methods are illegal and written permission, or a special collector's permit from the state's fish and game commission where the collecting is to be done, is required for their use.

Preservation by quick freezing in dry ice is most desirable. When this cannot be done, liquid preservatives are used. Fish over a half-pound or so should be injected with a syringe of preservative to prevent decomposition of internal organs.

1 Whole fish

Pesticide analyses of the whole fish yields data on gross contamination but cannot be used to determine cause of death. Quite high concentrations of pesticides are frequently found in fat deposits of apparently healthy fish.

2 Portion

Frequently, it is desired to know how much pesticide humans might ingest when eating fish. The fish is then divided into edible and non-edible portions. Since pesticides tend to be stored in the fat, and some of this may be cooked out of the fish during preparation, it may be necessary to analyze cooked fish to really get the answer.

3 Organ and tissue analyses

Analyses of individual organs and tissues yield information on the distribution of pesticides in the fish. This is prerequisite to understanding the biological implications of exposure of fish to pesticides. The metabolically active tissues are most informative.

Recent work, especially by Dr. Don Mount of RATSEC, on amounts of pesticide in blood of fish exposed to various concentrations, and for varying lengths of time, indicate that analyses of blood from dying fish may be a valuable method of determining probable cause of death where exposure to pesticides is suspected.

The blood is obtained from live fish by cutting off the tail at the caudal peduncle and collecting the blood in a small vial.

The blood is immediately quick frozen until analyses can be done.

V SAMPLES ARE WORTHLESS UNLESS ADEQUATELY LAPELLED

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This outline was prepared by G. J. Lauer, Associate Curator, Limnology Department, Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.

COMPARATIVE METHODOLOGY AND QUALITY CONTROL

I ROUND ROBIN, COLLABORATIVE OR COOPERATIVE STUDIES ARE UNDERTAKEN FOR A VARIETY OF MOTIVES

- A To accumulate data that may be used to determine the precision and accuracy of a new or modified test procedure.
- B To recheck an established procedure to ascertain whether there has been a deterioration in the accuracy arising from departures from prescribed routine.
- C To test the applicability of an established procedure to new materials.
- D To maintain a periodic check on the performance of a group of laboratories.

II DEFINITION OF TERMS

- A Series - A number of test results which possess common properties that identify them uniquely.
- B Mean - The sum of a series of test results divided by the number in the series. Arithmetic mean is understood.
- C Precision Data - Measurements which relate to the variation among the test results themselves - i.e., the scatter or dispersion of a series of test results, without assumption of any prior information. Sometimes referred to as indeterminate error or random error. The following measures apply:
 - 1 Variance - The sum of squares of deviations of the test results from the mean of the series after division by one less than the total number of test results.
 - 2 Standard Deviation - The square root of the variance.

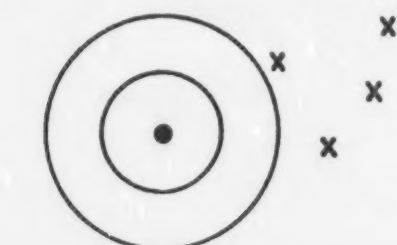
$$s = \sqrt{\frac{\sum (X_i - \bar{X})^2}{n - 1}}$$

X_i = value of single results

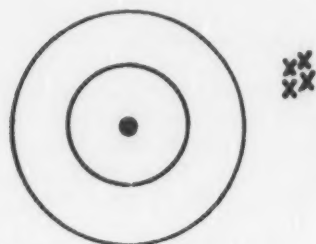
$\bar{X}$ = mean

n = number of results

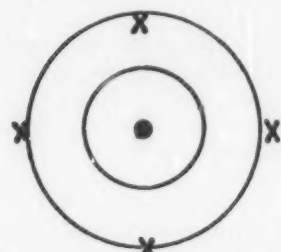
- 3 Relative Standard Deviation - The standard deviation of a series of test results as a percentage of the mean of this series. This term is preferred over "coefficient of variation."
- 4 Range - The difference in magnitude between the highest test result and the lowest test result in a series.
- D Accuracy Data - Measurements which relate to the difference between the average test results and the true result when the latter is known or assumed. The following measures apply:
 - 1 Mean Error - The average difference with regard to sign of the test results from the true results. Also equal to the difference between the average of a series of test results and the true results. Sometimes referred to as determinate error or bias which may result from:
 - a Incorrectly calibrated equipment.
 - b Method error.
 - c Personal mistakes.
 - 2 Relative Error - The mean error of a series of test results as a percentage of the true result.



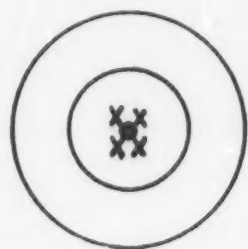
Inaccurate and Imprecise



Inaccurate, but Precise



Accurate, but Imprecise



Accurate and Precise

PRECISION AND ACCURACY

III COLLABORATIVE STUDY OF A METHOD

A The Test Method

- 1 Identical copies of a detailed description of all steps involved in the test method should be made available to all persons involved in the study.
- 2 In some cases, it may be desirable to evaluate various portions of the procedure as well as the over-all method. For example, in the analysis of pesticides: (See Attachment 1)
 - a The extraction and cleanup step.
 - b The determination step.
- 3 Should a single analysis or duplicate or triplicate analysis be required?

B The Test Sample

- 1 Necessary samples for the test should be supplied to each collaborator.
- 2 Generally, three samples providing for three levels of concentration, but concentrations should not be near limits of sensitivity or easily surmised values.
- 3 It is generally desirable to include both spiked samples and naturally contaminated samples.
- 4 In some cases, it may be necessary to supply additional samples containing varying but known amounts of interfering materials.

C Collaborating Laboratories

- 1 An indispensable condition for conducting a successful interlaboratory study is skill and familiarity with the method on the part of all participants.
- 2 For relatively new methods of test or methods that have been greatly modified, it is advisable to run a pilot study prior to the main interlaboratory test.

D Report Forms

- 1 The data report sheets should be identical blanks that are designed in an appropriate way for the easy recording of all raw data to be subjected to statistical analysis. (See Attachment 2)
- 2 Space should be provided for recording of any information pertinent to interpretation of the data.

E Statistical Analysis of Data

- 1 Usually requires the use of a desk calculator or a high speed computer.
- 2 Analysis of data should be carried out by person(s) conversant with statistical methods.
- 3 Interpretation of the results of a study is a joint responsibility of the statistician and a competent analyst who has working familiarity with the method under study. (See Table 1)

IV COMPARATIVE STUDY OF METHODS

- A A single sample or series of samples are analyzed by two or more methods (details of methods supplied to each collaborator) and the results are compared statistically

V QUALITY CONTROL

- A A round robin that takes in a cross section of typical laboratories goes beyond an evaluation of the procedure.
- 1 The data that will be collected reflect the merits of the procedure and also reflect the performance of the participating laboratories.
 - 2 Poor results may be caused by deficiencies in the procedure, failures to follow the procedure faithfully, poor technique on the part of the analyst, or poor reagents and standards.

Table 1

Sample A. 0.508 μg of Lindane
Per Liter of Water

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
1	0.228	.212	.044944
2	0.144	.296	.087616
3	0.470	.030	.000900
4	0.425	.015	.000225
5	0.512	.072	.005184
6	0.590	.150	.022500
7	0.258	.182	.033124
8	0.455	.015	.000225
9	0.228	.212	.044944
10	0.485	.045	.002025
11	0.398	.042	.001764
12	0.552	.112	.012544
13	0.568	.128	.016384
14	0.502	.062	.003844
15	0.542	.102	.020808
16	0.645	.205	.042025
17	0.098	.342	.116964
18	0.325	.115	.013225
19	0.600	.160	.025600
20	0.172	.268	.071824
21	1.500	rejected	-----
22	0.370	.070	.004900
23	0.642	.202	.040804
24	0.545	.102	.010404
25	0.605	.165	.027225
26	0.475	.035	.001225
27	0.555	.115	.013225
		11.389	.664452

$$\bar{X} = 0.44 \quad s = 0.16 \quad \text{Rel. Std. Dev. } 36.6$$

True Value 0.508 Mean Error 0.068
Rel. Error 13.4

- 3 Judgment of the procedure will be made on the data remaining after deleting absurd results.
- 4 By the use of standard samples and the standard addition techniques, each laboratory could carry out its own quality control program. If this were the case, the results would more truly represent solely an evaluation of the procedure.

B Rejection of Results and/or Laboratories

- 1 Control Charts - If the precision and accuracy of a method is known from previous studies or has been determined by the sponsoring or referee laboratories, then limits of normal variability can be set and the results of any laboratory falling outside of these limits can be rejected. (See Figure 1) Control limits for analysis are frequently set at ± 3 standard deviations.
- 2 Probability Curves - When the results reported by the individual laboratories are plotted on normal probability paper, a straight line should develop, if the data are normally distributed. If the data from a few laboratories do not fall on this straight line, they can be rejected. (See Figure 2)
 - a The accuracy and precision of the method can then be calculated from the remaining data.
- 3 Ranking of laboratory results
 - a Suppose nine laboratories analyzed five different samples.
 - b Assign the rank of one to the laboratory with the highest results, the rank of nine to the laboratory with the lowest result.
 - c If a tie exists, say for fifth place, assign the rank of 5.5 to the tied laboratories. If three are tied for fourth place, assign the middle rank of five to all three. This maintains the total of the rank at 45.
 - d When the laboratories have ranks assigned for all five materials, a score is given each laboratory by adding up its ranks. A score of five is the minimum possible, and a score of 45 the maximum.
 - e The corresponding five percent probability limits for various combinations of numbers of laboratories and number of materials can be calculated or determined from tables. For

9 laboratories and 5 samples, these limits are 9 and 41.

- f Laboratory scores exceeding these critical scores should be rejected. (See Table 2)

4 Testing for Extreme Mean (Table 3)

Briefly, the test involves computing a ratio which compares the distance of the suspected value from its neighbors to the range of all or most all of the observations. Reference of the ratio to a table of critical values for test ratios indicates whether such a large ratio is likely on the basis of chance. If the ratio is greater than or equal to the critical value the probability is small that the suspected outlier is from the sample distribution, hence the outlier is rejected.

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- 1 ASTM Committee E-11, ASTM Manual for Conducting an Interlaboratory Study of a Test Method. ASTM Special Technical Publication No. 335. American Society for Testing Materials, 1963.
- 2 Youden, W. J. Ranking Laboratories by Round-Robin Tests. Material Research and Standards 3, 9. 1963.
- 3 Youden, W. J. The Collaborative Test. J. Assoc. Offic. Agri. Chemists 46, 55. 1963.
- 4 Horwitz, W. Chemists Guidance Manual. U.S. Department of Health, Education, and Welfare, Food and Drug Administration. August 1964.
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Table 3*

Testing for Extreme Mean

Statistic	No. of obs., k	Critical values						
		$\alpha = .30$	$\alpha = .20$	$\alpha = .10$	$\alpha = .05$	$\alpha = .02$	$\alpha = .01$	$\alpha = .005$
$r_{10} = \frac{X_2 - X_1}{X_k - X_1}$	3	.684	.781	.886	.941	.976	.988	.994
	4	.471	.560	.679	.765	.846	.889	.926
	5	.373	.451	.557	.642	.729	.780	.821
	6	.318	.386	.482	.560	.644	.698	.740
	7	.281	.344	.434	.507	.586	.637	.680
$r_{11} = \frac{X_2 - X_1}{X_{k-1} - X_1}$	8	.318	.385	.479	.554	.631	.683	.725
	9	.288	.352	.441	.512	.587	.635	.677
	10	.265	.325	.409	.477	.551	.597	.639
$r_{21} = \frac{X_3 - X_1}{X_{k-1} - X_1}$	11	.391	.442	.517	.576	.638	.679	.713
	12	.370	.419	.490	.546	.605	.642	.675
	13	.351	.399	.467	.521	.578	.615	.649
$r_{22} = \frac{X_3 - X_1}{X_{k-2} - X_1}$	14	.370	.421	.492	.546	.602	.641	.674
	15	.353	.402	.472	.525	.579	.616	.647
	16	.338	.386	.454	.507	.559	.595	.624
	17	.325	.373	.438	.490	.542	.577	.605
	18	.314	.361	.424	.475	.527	.561	.589
	19	.304	.350	.412	.462	.514	.547	.575
	20	.295	.340	.401	.450	.502	.535	.562
	21	.287	.331	.391	.440	.491	.524	.551
	22	.280	.323	.382	.430	.481	.514	.541
	23	.274	.316	.374	.421	.472	.505	.532
	24	.268	.310	.367	.413	.464	.497	.524
	25	.262	.304	.360	.406	.457	.489	.516

* Data taken from Introduction to Statistical Analysis by Dixon & Massey, p. 412.

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- 7 Guides for Measures of Precision and
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- 8 Accuracy in Clinical Chemistry. Dade
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- 9 Dixon, W. J. and Massey, F. J. Intro-
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2nd Edition. McGraw-Hill Book Co.,
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This outline was prepared by Earl F.
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FIGURE 1. CONTROL CHART

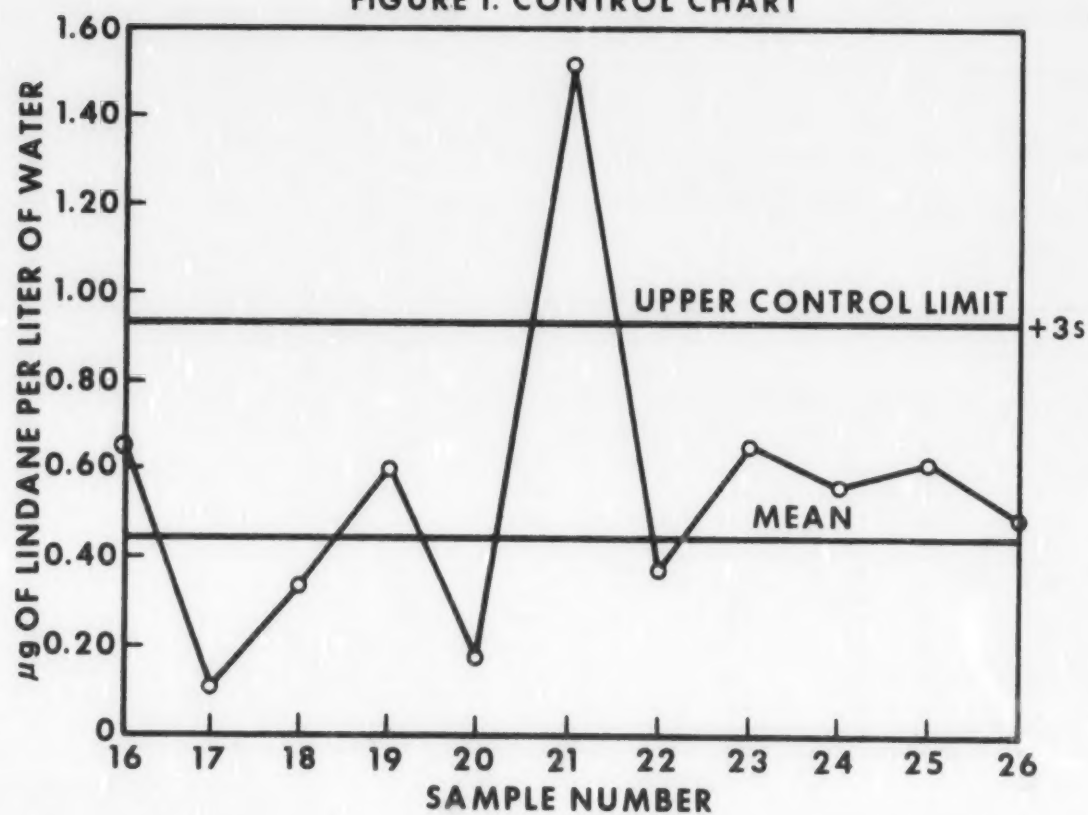


FIGURE 2. PROBABILITY CURVE

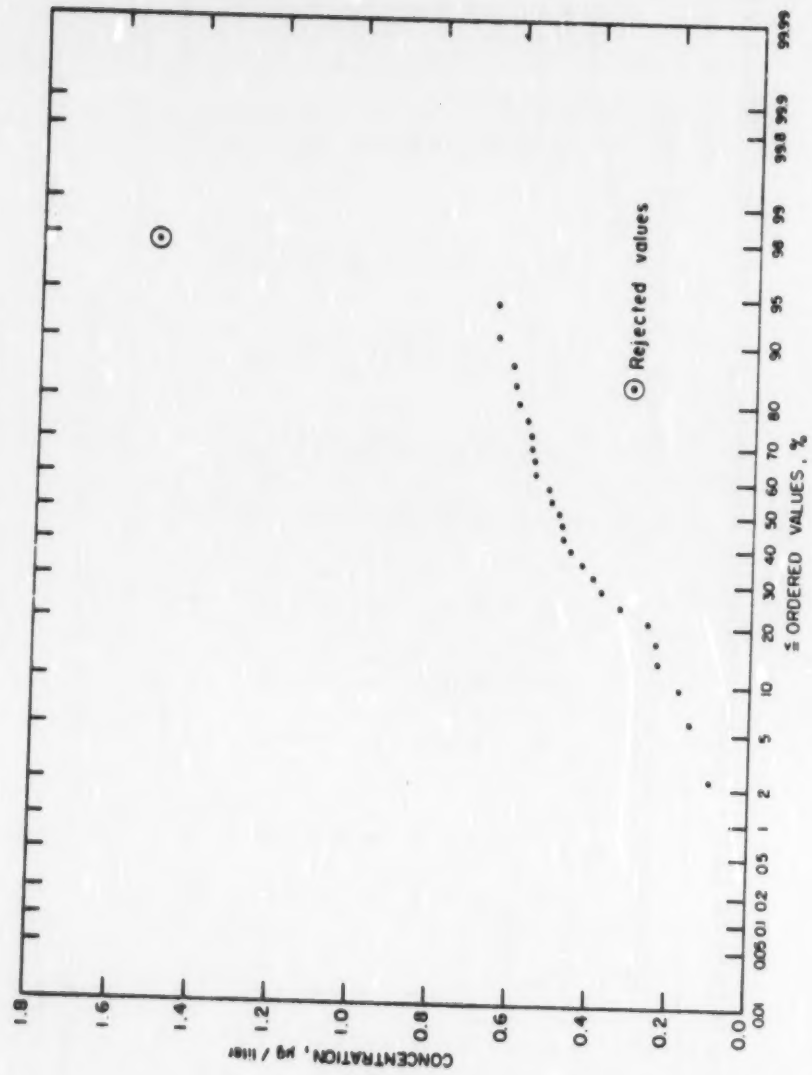


Table 2
RANKS AND SCORES OF LABORATORIES REPORTING PERCENTAGE OF TOTAL ALKALOIDS AS NICOTINE^a

Collaborator's Choice of Method						Tentative Recommended Method						
Sample						Sample						
No.	A	B	C	D	E	Score ^b	A	B	C	D	E	Score ^c
1	...	...	...	...	...	...	6	5	7	10	2.5	30.5
2	1	1	2	2	3	9	2	3	1	6	5	17
3	9	9	7	7	8	40	7	8	4	7	10	36
4	7.5	8	9	6	7	37.5	8	6.5	5.5	3	4	32
5	...	...	...	...	...	...	3	1.5	5.5	3.5	1	14.5
6	6	2	4.5	1	1.5	15	4	5.5	9	1	7.5	28
9	4	4	4.5	4	5	21.5	10	10	10	9	9	48
10	2	3	1	3	1.5	10.5	...	...	...	...	...	...
11	5	6	6	8	5	30	1	1.5	2	2	2.5	9
12	7.5	6	8	9	9	39.5	...	...	...	...	...	...
12a	...	...	...	...	...	...	9	9	8	3.5	7.5	37
13b	...	...	...	...	...	...	5	4	3	5	6	23
15	3	6	3	5	5	22	Average....27.5					
						Average....25.0						

^a See Journal, Assn. of Official Agricultural Chemists, Vol. 42 p 305, 1959

^b Critical scores are 9 and 41.

^c Critical scores are 10 and 45.

Attachment 1

ANALYTICAL REFERENCE SERVICE

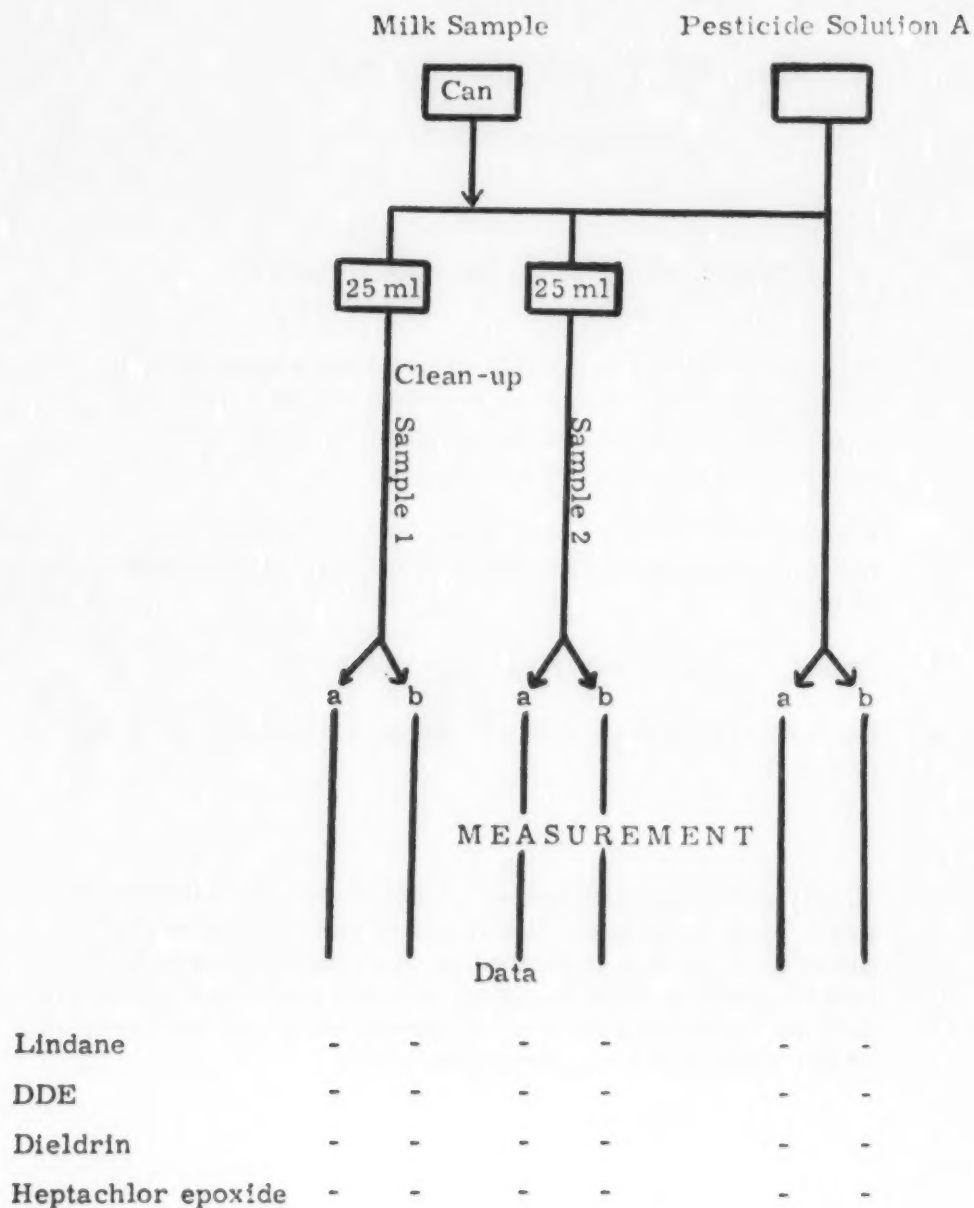
Pesticides in Milk

Directions for Preparing Milk Samples*

1. To each of two 25 ml volumetric flasks carefully add by pipet, 1.0 ml of pesticide solution A and make up to a volume of 25 ml by the addition of evaporated milk from the can. Label the flasks A-1 and A-2.
2. Blend the mixture in each flask well. Extract and clean up each entire sample and do duplicate assays of each.
3. Repeat the above two steps with Sample B.
4. Do duplicate assays of the remaining pesticide solutions A and B.

* Kindly note that the manner of preparing the mixture of pesticides in milk and the volumes specified are slightly different than was indicated on our announcement form. These changes were made on the basis of some recommendations (resulting from prior experience with such studies) of the Food and Drug Administration.

DIAGRAM OF SAMPLING PLAN



The above plan is to be repeated with Sample B.

Attachment 2

ANALYTICAL REFERENCE SERVICE
Pesticides in Milk

Date Analyzed _____

ARS CODE NO. _____

Laboratory _____

Pesticide Solution *

SAMPLE A ($\mu\text{g/ml}$)	Lindane	DDE	Dieldrin	Heptachlor Epoxide
Assay 1	_____	_____	_____	_____
Assay 2	_____	_____	_____	_____
SAMPLE B ($\mu\text{g/ml}$)				
Assay 1	_____	_____	_____	_____
Assay 2	_____	_____	_____	_____

Pesticides in Milk **

SAMPLE A ($\mu\text{g/ml}$)	Lindane	DDE	Dieldrin	Heptachlor Epoxide
<u>Extraction and Clean-up 1</u>				
Assay a	_____	_____	_____	_____
Assay b	_____	_____	_____	_____
<u>Extraction and Clean-up 2</u>				
Assay a	_____	_____	_____	_____
Assay b	_____	_____	_____	_____
SAMPLE B ($\mu\text{g/ml}$)				
<u>Extraction and Clean-up 1</u>				
Assay a	_____	_____	_____	_____
Assay b	_____	_____	_____	_____
<u>Extraction and Clean-up 2</u>				
Assay a	_____	_____	_____	_____
Assay b	_____	_____	_____	_____

*Report your results to the nearest hundredth of a microgram.

**Report your results to the nearest thousandth of a microgram per ml/ μg
of mixture of pesticides in milk.

SUPPLEMENTARY INFORMATION NEEDED TO EVALUATE
THE RESULTS STATISTICALLY

1. Extraction and Clean-up Method used

a. Name of Method _____

b. Reference(s)* _____

2. Determination

a. Electron capture gas chromatography _____

(1) Column packing _____

(2) Temperature of column _____

b. Micro-coulometric gas chromatography _____

(1) Column packing _____

(2) Temperature of column _____

c. Thin-layer chromatography _____

(1) Absorbent _____

(2) Developing solvent(s) . _____

d. Other Method _____

(1) _____

(2) _____

DIFFICULTIES OR COMMENTS:

*If unpublished please submit details.

OTHER METHODS USED IN PESTICIDE RESIDUE ANALYSIS

I INTRODUCTION

One consideration in the choice of an extraction and clean-up technique in pesticide analysis is the fat content of the sample. Table 1 lists some characteristic compositions of food samples.

Table 1.¹ COMPOSITION OF FOOD SAMPLES

Substrate	% water	% fat
Butter	15.5	81
Milk, pasteurized	87.2	3.7
Strawberries	89.9	.5
Eggs, raw whole	73.7	11.5
Catfish (edible portion)	78.0	3.1
Peas	11.7	1.3
Grasses	60 - 80	.5 - 1.3

¹ Reproduced with permission from Pesticide Analytical Manual, U.S. Department of Health, Education, and Welfare, Food and Drug Administration, July, 1965.

II NON-FATTY SAMPLES

Non-fatty samples are those considered to have 2% or less fat. The Mills method⁽⁵⁾ is an established method used by the Food and Drug Administration for the analysis of chlorinated hydrocarbons in such samples.

A Extraction

A representative aliquot of the sample is blended with acetonitrile for 1 - 2 minutes in a Waring blender. The acetonitrile filtrate contains the pesticides, pigments, and other organic impurities.

B Partition

The acetonitrile filtrate is further cleaned up by an acetonitrile-water-petroleum ether partition. In the partition pesticides and non-polar interfering substances are forced into the petroleum ether layer while polar interfering substances remain behind in the aqueous layer.

C Sorption

1 Further clean-up of pesticides from non-polar interfering substances may be required on a florisil column. The Food and Drug Administration procedure requires that the column be eluted successively with 6% diethyl ether in petroleum ether and 15% diethyl ether in petroleum ether. The pesticides appearing in each eluate are shown in Table 2.

Table 2. SEPARATION OF PESTICIDES ON FLORISIL COLUMN

<u>6% Diethyl ether: petroleum ether</u>	<u>15% Diethyl ether: petroleum ether</u>
Aldrin	Dieldrin
Lindane	Endrin
Chlordane	
DDE	
DDT	
Heptachlor	
Heptachlor epoxide	
Methoxychlor	
Toxaphene	

2 It may be necessary to further clean up the 15% fraction on a magnesium

oxide-celite column using petroleum ether as the eluent especially if paper chromatography is used as the determinative step.

D Determinative Step

The extract should now be ready for the determinative step of gas chromatography, thin layer chromatography, paper chromatography or a combination of these.

III FATTY SAMPLES

Fatty samples are those considered to have more than 2% fat.

A Mills Procedure

Mills has also developed an established method^(3,4) used by the Food and Drug Administration for the analysis of chlorinated hydrocarbons in such samples.

1 Extraction

Pesticides are found in the lipid fraction of the fatty sample. Therefore, the first step of the Mills method is to isolate the lipid fraction from proteinaceous and other water soluble components by the combined action of methyl or ethyl alcohol and the fat solvent diethyl ether. The solvents and sample are mixed together in a centrifuge bottle (in the case of a liquid sample such as milk) or a Waring blender (in the case of a solid sample).

2 Partition, sorption, and determinative steps

The fat extract is taken up in hexane, partitioned into acetonitrile and then cleaned up by the same partition and sorption methods as described for non-fatty samples. The sample is then ready for the appropriate determinative step.

B Saponification

Fatty samples can be cleaned up rapidly by saponification either as a primary method

or in combination with other partition and sorption methods.⁽⁶⁾ If used as a primary method, clean-up may not be total. The method must be used for compounds stable to alkali (e.g., aldrin, dieldrin and endrin) or compounds which undergo quantitative conversion to known derivatives under alkaline conditions (e.g., the conversion of DDT to DDE).

- 1 The sample is heated with alcoholic potassium hydroxide to convert the triglycerides to soaps and glycerine.
- 2 The soap solution is then extracted with a solvent such as hexane. The pesticides are partitioned into the hexane, while the soaps and other water soluble compounds remain in the aqueous layer.
- 3 The extract is then ready for the determinative step.

IV CONCLUSION

The Mills' methods for the extraction and clean-up of fatty and non-fatty samples are presented. Saponification clean-up is also described.

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This outline was prepared by B.A. Punghorst,
former Chemist, Training Branch, Ohio
Basin Region, FWPCA, SEC.

DETERMINATION OF CHOLINESTERASE INHIBITION IN FISH BRAIN

I INTRODUCTION

A Principle

The toxicity of organic phosphorus insecticides to fish life, as determined by standard bioassay procedures, is very high. Although generally not as toxic as the chlorinated hydrocarbons, some of the organic phosphorus compounds have a 96-hour TL₅₀ as low as 0.2 mg/l (Henderson and Pickering, 1957, Hoffman, 1957).

The development of the organic phosphorus insecticides has had an unusual history in that the mode of action had an early elucidation and has been an extremely fruitful area of investigation for toxicological studies (Metcalf, 1959). The toxic action in insects results from inactivation of the enzyme cholinesterase. This inactivation has been shown to occur in the brain of fish following exposure to the organic phosphorus compounds (Weiss, 1958, 1959).

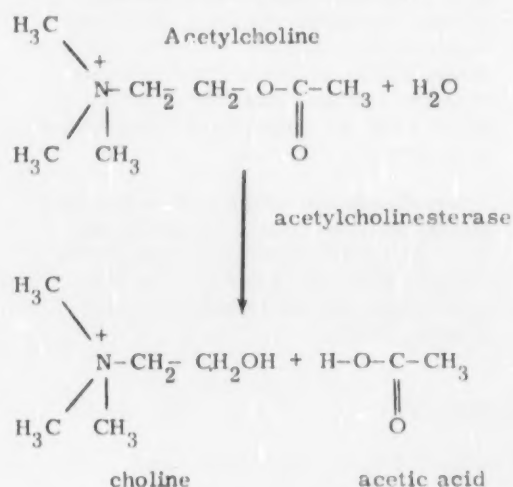
The brain is essentially nerve tissue and is rich in the true cholinesterases as compared to the pseudo-cholinesterases found in blood (Whittaker, 1951). In most species of fish the brain is readily accessible and is easily removed in toto. The brain is supplied with oxygenated blood that comes directly from the gills. The transfer, therefore, of toxic materials from the water across the gill membranes and to the reaction site is quick and direct.

The brain cholinesterase activity is determined using acetylcholine chloride as the substrate. Acetylcholine is the material destroyed in vivo by the action of cholinesterase at nerve fiber junctions. A brain homogenate with a tissue concentration of 5 mg/ml in a "Tris" buffer at pH 8.2 is incubated with 0.004 M

acetylcholine chloride at 25°C for 20 minutes. The residual acetylcholine is determined colorimetrically by the Hestrin method (Hestrin, 1949). The brain acetylcholinesterase (AChE) activity is reported as micromoles of acetylcholine hydrolyzed per milligram (wet weight) of brain tissue per hour.

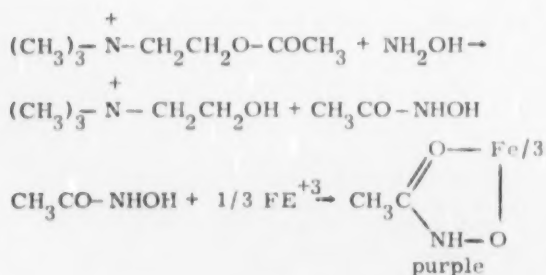
The reactions occurring in this analytical procedure are given below.

- 1 The acetylcholine substrate is hydrolyzed by the acetylcholinesterase enzyme in the brain tissue.



- 2 The acetylcholine remaining after the enzymatic reaction is reacted with alkaline hydroxylamine and excess ferric ion to yield the purple color of ferric acethydroxamate.

Determination of Cholinesterase Inhibition in Fish Brain



B Interferences

Esters of carboxylic acids and certain other substances may also yield a purple color and thus interfere with this method.

C Sampling and Storage

- 1 In case of a fish kill where pesticides of organophosphorus nature are suspected, sampling should be such that 1) distressed, but not dead fish are taken 2) control fish are of the same size and species, preferably from same area, i.e. upstream from source of pollution.
- 2 When sample specimens are taken they should be immediately frozen in dry ice. At -25°C there is no significant change in enzyme activity of frozen fish for as long as 3 months storage (Weiss, 1961).

II REAGENTS

Tris buffer: 0.2 m "Tris" (24.2 gm)
0.2 m NaCl (11.69 gm)
0.02 m MgCl_2 (4.06 gm)

To one liter. Store in refrigerator.
Adjust to pH of 8.2 with HCl.

Color Reagents

1. HCl - 4.0 N 333 ml of con. (12 N) HCl to 1 liter
2. NaOH - 3.5 N 140 gm NaOH/liter
3. NH_2OH - HCl - 2.0 M 27.8 gm/200 ml
 H_2O - Store in refrigerator

4. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 100 gm/l of 0.1 N HCl

5. NaAc - 0.001 M 82 mg/l of anhydrous sodium acetate

Substrate

Acetylcholine Chloride - 0.004 m

73.0 mg Acetylcholine chloride/100 ml
0.001 M NaAc. Adjust pH to 4.5. Store in refrigerator.

III STANDARD CURVE

- A Pipette 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of substrate into an 18 x 175 mm test tube.
- B Add 1.0, 0.8, 0.6, 0.4, 0.2, and 0 ml of the 0.001 m NaAc buffer to the respective tubes to give a total volume of 1.0 ml in each tube.
- C Add 1.0 ml of "tris" buffer to each tube.
- D Add 2.0 ml of Alkaline hydroxylamine hydrochloride (mix color reagents 2 and 3 1:1) shake.
- E Add 1.0 ml of 4.0 N HCl and shake.
- F Add 1.0 ml of FeCl_3 solution and shake.
- G Read at 540 millimicrons using first tube as a blank.

IV SAMPLE PROCEDURE - Do Steps 1-3 as quickly as possible.

- A Remove brain from the fish and weigh on previously tared weighing pan.
- B Transfer brain from the pan to the homogenizer. Add 4.0 ml "Tris Buffer". Part of the 4.0 mls can be used to rinse any tissue adhering to weighing pan.

- C After tissue has been homogenized, transfer homogenate to an 18 × 175 mm test tube. Add "Tris Buffer" until the ratio of tissue to buffer is 5.0 mg tissue/1.0 ml buffer. Place tube of homogenate in an ice bath. The remainder of the analysis must be completed the same day.
- D Pipette three 1.0 ml portions of the tissue homogenate into three separate 18 × 175 test tubes. Label the first tube blank and the others S₁ and S₂.
- E Add 1.0 ml portions of substrate to each tube at carefully timed intervals. Mix thoroughly.
- NOTE: Rate of reactions between enzyme and substrate is extremely sensitive to variations in time and temperature.
- F Immediately place in constant temperature water bath. Hold at 30°C for 20 minutes.
- G Exactly 20 minutes after the addition of substrate add 2.0 ml of alkaline hydroxylamine hydrochloride to tubes S₁ and S₂. Shake these tubes vigorously and place in ice bath.
- H Add 1.0 ml of 4.0 N HCl to the blank, S₁ and S₂.
- I Add 2.0 ml alkaline hydroxylamine hydrochloride to the blank.
- J Add 10 ml ferric chloride solution to each tube.
- K Add from 0.5 to 1.0 gram of Celite 545 and shake vigorously. Before mixture settles filter it into clean tubes or spectrophotometric cuvettes. Use no. 42 Whatman filter paper.
- L With wavelength setting at 540 mμ, insert tube marked "blank" and adjust absorbance to read zero. Read and record absorbance of S₁ and S₂.

V CALCULATIONS

- A Using the prepared standard curve and absorbance readings obtained from the spectrophotometer, determine micromoles acetylcholine chloride (Ach) remaining.
- B Calculate Ach hydrolyzed. Ach hydrolyzed/20 min/3 mg equals 40 micromoles minus micromoles Ach remaining.
- C Multiply by 3/5 to convert to standard form of Ach hydrolyzed/ hour/mg brain tissue (specific activity).

VI Example of tabulation of data from controls and distressed fish kill where organophosphorus pesticide is suspected.

VII CALCULATION OF PERCENT INHIBITION FROM DATA FROM TABLE 1

- A Average the specific activity of distressed fish from Table 1. 0.55 Ach/hr/mg
- B Average the specific activity of controls from Table 1. 1.66 Ach/hr/mg
- C Subtract the specific activity of distressed fish from controls and determine activity inhibited.
- D Divide activity inhibited by activity of controls to determine percent inhibition.

Example:
$$\begin{array}{r} 1.66 \\ 0.55 \\ \hline 1.11 \text{ activity inhibited} \end{array}$$

$$\frac{1.11}{1.66} = 67\% \text{ inhibition of enzyme activity}$$

Determination of Cholinesterase Inhibition in Fish Brain

TABLE 1

Distressed Fish

<u>Length</u>	<u>Weight</u>	<u>Brain Wt. mg</u>	<u>O. D.</u>	<u>Ach/hr/mg</u>
4 1/2"	21.5 g	49.5	.54	0.51
4 "	19.1 g	49.5	.52	0.58

Control Fish

<u>Length</u>	<u>Weight</u>	<u>Brain Wt. mg</u>	<u>O. D.</u>	<u>Ach/hr/mg</u>
3 3/4"	18.5 g	51.5	.24	1.58
4 "	18.7 g	71.5	.18	1.77

REFERENCE

Weiss, C.M. Physiological Effect of Organic Phosphorus Insecticides on Several Species of Fish. Trans. of the Am. Fish Soc. 90:143-152. 1961

This outline was prepared by E. William Loy, Chemist, Analytical Services, Southeast Water Laboratory, Athens, GA.

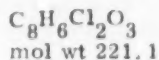
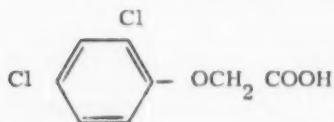
ANALYSIS OF WATER FOR 2,4-D

I OBJECTIVE

Given a water sample containing the herbicide 2,4-D, the student will identify and quantitate the amount present. He will apply the proper techniques of extraction, esterification, and gas chromatography to complete the analysis.

2,4-D

2,4-dichlorophenoxyacetic acid



II EQUIPMENT

A Glassware

- 1 100 ml graduate cylinder
- 2 50 ml graduate cylinder (2)
- 3 2000 ml beaker
- 4 400 ml beaker
- 5 150 ml beaker (4)
- 6 2000 ml separatory funnel
- 7 125 ml separatory funnel
- 8 Chromatographic column
- 9 Kuderna-Danish assembly
- 10 5.0 ml mohr pipette (2)
- 11 1.0 ml mohr pipette
- 12 Centrifuge tube

B Reagents

- 1 1:1 sulfuric or hydrochloric acid
- 2 Sodium sulfate (anhydrous)

- 3 Sodium sulfate solution (saturated)
- 4 Boron trifluoride-methanol solution 14% W/V.
- 5 Methylene chloride - redistilled in glass.
- 6 Methanol - redistilled in glass.

C Miscellaneous

- 1 Solvent containers
- 2 Waste solvent containers
- 3 Thermometers 0 - 100°C
- 4 Rinse bottles
- 5 Hot water bath
- 6 Steam bath
- 7 Cork rings
- 8 Dry air system
- 9 Syringe
- 10 Gas chromatograph

III METHOD OF ANALYSIS

A Extraction

- 1 Measure and transfer 1 liter of the water sample to a 2 liter separatory funnel.
- 2 Add 2 ml 1:1 HCl per liter of sample. pH should be 2.3 or lower.
- 3 Add 60 ml of methylene chloride. Stopper, invert and release pressure through stopcock.
- 4 Continue to shake flask for two minutes releasing pressure as necessary.
- 5 After allowing fractions to separate, drain the lower water layer into a 2 liter beaker. Then drain the remaining solvent layer into a 400 ml beaker.

- 6 Transfer the water from the 2 liter beaker back into the separatory funnel. Repeat steps 3, 4, and 5.
- 7 Add 60 ml methylene chloride. Stopper and shake separatory funnel releasing pressure as necessary.
- 8 Allow the fractions to separate and then drain the lower water layer into a 2 liter beaker. This water may now be discarded. Drain the remaining solvent layer into the 400 ml beaker containing the two previous extractions.

B Drying of Hexane Extract

- 1 Place a plug of extra fine Pyrex glass wool in the bottom of a 27 mm ID x 150 mm crucible holder (filter tube).*
- 2 Saturate the glass wool with methylene chloride.
- 3 Carefully pour all the sample extract except emulsion into the filter tube.
- 4 Quantitatively rinse beaker, pouring rinsings but not emulsion into the filter tube.
- 5 As solvent reaches level of glass wool in the filter tube, slowly pour emulsion down side of tube.
- 6 Rinse sides of the tube with a small amount of methylene chloride.

C Esterification

- 1 Using a hot water bath and a stream of dry air, very carefully evaporate the dried extract to a volume of approximately 5 ml.
- 2 Quantitatively transfer the extract to an uncalibrated Kuderna-Danish tube. Add 2 ml of methanol.

- 3 Place the Kuderna-Danish tube containing the extract into a beaker of water previously adjusted to 30 - 35°C. Hold beaker at this temperature while using dry air to concentrate extract to approximately 1 ml.

- 4 Add 2 ml of BF₃ in methanol to the tube containing sample extract.

- 5 Connect the Kuderna-Danish tubes to the Kuderna-Danish evaporators (use spring clips); attach Snyder column, add a small boiler, and suspend in the steam bath for 2 minutes.

- 6 Remove the Kuderna-Danish apparatus from the steam bath and allow to cool.

- 7 Remove the Snyder column. Quantitatively transfer the contents of the K. D. tube to a 125 ml separatory funnel using three 10 ml portions of petroleum ether and two 10 ml portions of distilled water. Wash down the sides of the apparatus with each rinse.

- 8 Shake the separatory funnel for 2 minutes. Allow the layers to separate. Drain the water layer off the bottom and discard it. Quantitatively transfer the ether layer to a 150 ml beaker.

- 9 Quantitatively transfer the ether extract to a 100 ml volumetric flask. Avoid transferring any of the water droplets adhering to the bottom or sides of the beaker. Bring to volume with petroleum ether.

D Determinative Steps

Inject into a gas chromatograph equipped with an electron capture detector.

*NOTE: Anhydrous sodium sulfate is not used because it causes the herbicides to plate out resulting in a poor yield.

This outline was prepared by E. William Loy, Chemist, Analytical Services, and William R. Davis, former Chemist, Training Section, Southeast Water Laboratory, Athens, Ga.

ANALYSIS OF FISH AND RELATED BIOLOGICAL SPECIMENS
FOR ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDES

I REAGENTS AND SPECIAL EQUIPMENT

- A Solvents - Petroleum ether, ethyl ether, methylene chloride, hexane, acetonitrile. Burdick and Jackson "Distilled in Glass" or the equivalent.
- B Florisil, PR grade, 60/100 mesh.
- C Sodium sulfate, anhydrous, granular, reagent grade. Heat at 500°C for 2 hours.
- D Dry ice - free of contamination.
- E Sodium chloride solution - 2%. (Pre-extract with methylene chloride).
- F Waring Blender. Enlarge the vent in the cover to 1/2 inch.
- G Chromatographic column - 400 mm x 20 mm with a Teflon stopcock.
- H Kuderna - Danish evaporators.
- I Separatory funnels - 125 ml and 500 ml.
- J Pyrex glass wool.

II SAMPLE PREPARATION

- A Whole fish analysis - Chop the sample as fine as possible.

Edible portion - Skin and filet enough muscle tissue to provide 25-100 gm of sample. Chop into 1/2 inch chunks.
- B Add about 1/2 inch of chopped up dry ice to a Waring blender chamber. Add the chopped fish (25-100 gm is the ideal sample size) and an additional portion of chopped dry ice. Blend until the contents are finely divided. The addition of more dry

ice may be necessary to maintain an excess of dry ice. Transfer the blended dry ice and sample to a piece of aluminum foil, wrap loosely and immediately place in a freezer. Store overnight to allow the CO₂ to sublime out of the sample

1 Solvents for column extraction

- a organochlorine pesticides - cyclohexane
- b organophosphorus pesticides - 10% ethyl ether in petroleum ether
- c triazines and PCP - ethyl ether
- d carbamates - 5% methanol in ether
- e phenoxy acid herbicides - 1% H₃PO₄ in acetone

- C Weigh about 40 gm of anhydrous sodium sulfate into a 250 ml beaker.

If sample size permits, weight 20.0 gm of the powdered frozen fish into the beaker. Immediately add an additional portion of anhydrous sodium sulfate to the beaker to provide a 4:1 mixture of sodium sulfate to fish and mix as thoroughly as possible with a spatula.

NOTE: The powdered frozen fish sample thaws very rapidly, therefore, this step must be carried out as quickly as possible to prevent the formation of lumps.

- D Allow the sample to set for one hour, with frequent stirring to provide a fairly free flowing powder.

III EXTRACTION

- A Prepare a chromatographic column by inserting a small plug of glass wool in the bottom and covering this with 1/2

inch of anhydrous sodium sulfate. (Pre-rinse with two 50 ml portions of methylene chloride). Transfer the sample mixture to the column and rinse the beaker and wall of the column with petroleum ether. Extract the sample by eluting the column with 190 ml of 10% ethyl ether in petroleum ether. Maintain a flow rate of 3-6 ml/min.

- B Concentrate the extract to about 5 ml in a beaker using a gentle stream of N_2 .

IV HEXANE-ACETONITRILE PARTITIONING

- A Transfer the extract to a 125 ml separatory funnel and bring to 15 ml volume with hexane. Have the separatory funnel marked at 15 ml.
- B Add 30 ml of acetonitrile saturated with hexane to the separatory funnel. Gently mix solvents in separatory funnel by rocking back and forth a few times (CAUTION: Release pressure in separatory funnel by opening stopcock).
- C Extract pesticides into acetonitrile by shaking separatory funnel for 1 minute.
- D Drain the lower layer (acetonitrile) into a 1L separatory funnel containing 700 ml of 2% NaCl solution (Pre-extract the NaCl solution with two 50 ml portions of methylene chloride). Re-extract with 3 additional 30 ml portions of redistilled acetonitrile saturated with hexane. Collect all acetonitrile extracts in the 1 liter separatory funnel.
- E Add 100 ml of hexane and shake the funnel for 30 seconds.
- F Drain the aqueous layer into a 1 liter beaker and the hexane into a 500 ml separatory funnel. Transfer the

aqueous layer back to the 1 liter separatory funnel and shake 30 seconds with an additional 100 ml of hexane.

- G Discard the aqueous layer and combine the hexane layers. Wash the combined hexane extracts with two 100 ml portions of distilled water, discarding the wash water.
- H Dry the extract by passing it through a pad of pyrex glass wool pre-rinsed with 50 ml of methylene chloride and 50 ml of hexane.
- I Concentrate to 10-20 ml using a Kuderna-Danish evaporator.
- J Clean up the extract by using Florisil column chromatography.
- K Analysis is by Gas Chromatography.

REFERENCE

- 1 Hesselberg, R. J. and Johnson, J. L.,
Bull. of Environ. Contam. Toxicology, Vol. 7, No. 213, 115-120 (1972).

Descriptors: Organic Compounds Pesticides, Chlorinated Hydrocarbon Pesticides, Organo Phosphorous Pesticides, Techniques Analytical, Chromatography, Gas Chromatography

SEDIMENT EXTRACTION PROCEDURE

I EQUIPMENT AND CHEMICALS

- A Pans, approximately 14"x10"x2 1/2".
- B Oven, drying.
- C Muffle Furnace.
- D Desiccator.
- E Crucibles, porcelain, squat form, size 2.
- F Omni-mixer and 400 ml chambers.
- G Chromatographic columns, 400 mm x 22 mm with teflon stopcock.
- H Separatory funnels, 500 ml and 250 ml with teflon stopcocks.
- I Filter tube, 180 mm x 25 mm.
- J Pyrex glass wool - pre-extracted with methylene chloride in a soxhlet extractor.
- K Sodium sulfate, anhydrous, Baker, prerinsed with methylene chloride.
- L n-Hexane, Burdick and Jackson "Distilled-in-glass" or the equivalent.
- M Acetone, Burdick and Jackson "Distilled-in-glass" or the equivalent.
- N Methylene chloride, Burdick and Jackson "Distilled-in-glass" or the equivalent.
- O Acetone-hexane, 1:1.
- P Distilled water, suitable for pesticide residue analysis.
- Q Sodium sulfate solution, saturated.
- R Methylene chloride-hexane, 15% v/v.

II PROCEDURE

- A Decant and discard the water layer over the sediment. Mix the sediment to obtain as homogenous a sample as possible and transfer to a pan to partially air dry for about 3 days at ambient temperatures.

NOTE: Drying time varies considerably depending on soil type and drying conditions. Sandy soil will be sufficiently dry in one day whereas muck requires at least three days. The silt and muck sediment is sufficiently dry when the surface starts to split but there should be no dry spots. Moisture content will be 50-80% at this point.

- B Weigh 50 gm of the partially dried sample into a 400 ml Omni-Mixer chamber. Add 50 gm of anhydrous sodium sulfate and mix well with a large spatula. Allow to stand with occasional stirring for approximately one hour.
- C Immediately after weighing the sample for extraction weigh approximately 5 gm of the partially dried sediment into a tared crucible. Determine the percent solids by drying overnight at 103°C. Allow to cool in a desiccator for half an hour before weighing. Determine the percent volatile solids by placing the oven dried sample into a muffle furnace and igniting at 550°C for 60 minutes. Allow to cool in a desiccator before weighing.
- D Attach the 400 ml chamber to an Omni-Mixer and blend for about 20 seconds. The sample should be fairly free flowing at this point.
- E Carefully transfer the sample to a chromatographic column. Rinse the 400 ml chamber with small portions of hexane adding the rinsings to the column.
- F Elute the column with 250 ml of 1:1 acetone-hexane at a flow rate of 3-5 ml/min using a 400 ml beaker or a receiver.

- G Concentrate the sample extract to about 100 ml and transfer to a 500 ml separatory funnel containing 300 ml of distilled water and 25 ml of saturated sodium sulfate solution. Shake the separatory funnel for two minutes.
- H Drain the water layer into a clean beaker and the hexane layer into a clean 250 ml separatory funnel.
- I Transfer the water layer back into the 500 ml separatory funnel and re-extract with 20 ml of 15% methylene chloride in hexane, again shaking the separatory funnel for two minutes. Allow the layers to separate. Discard the water layer and combine the solvent extracts in the 250 ml separatory funnel.
- J Wash the combined solvent extract by shaking with 100 ml of distilled water for 30 seconds. Discard the wash water and re-wash the extract with an additional 100 ml of distilled water again discarding the wash water.
- K Pass the solvent extract through a drying column packed with an inch of glass wool. Concentrate the dried extract to about 10 ml.
- L The sample is now ready for Florisil clean-up followed by analysis by gas chromatography.

III CALCULATIONS

A Percent Dry Solids

$$\frac{\text{gm of dried sample} \times 100}{\text{gm of sample}} = \% \text{ Dry Solids}$$

B Percent Volatile Solids

$$\frac{\text{gm of dried sample} - \text{gm of ignited sample}}{\text{gm of volatile solids}}$$

$$\frac{\text{gm of volatile solids} \times 100}{\text{Volatile Solids}} = \frac{\text{gm of sample}}{\text{gm of sample}}$$

C Concentration of Pesticide in Sediment

$$\frac{\% \text{ dry solids} \times 50 \text{ gm}}{\text{sample extracted}} = \text{gm of dry sample extracted}$$

$$\frac{\text{ul of sample extract injected} \times \text{gm of of dry sample extracted}}{\text{sample injected}} = \frac{\text{gm of dry sample extracted}}{\text{ul of sample extract}}$$

$$\frac{\text{ng of pesticide}}{\text{gm of dry sample injected}} = \text{ppb of pesticide}$$

Descriptors: Organic Compounds Pesticides, Techniques Analytical, Chromatography, Gas Chromatography, Sediments

SAMPLING FOR PESTICIDES AND GENERAL ORGANIC ANALYSIS

I WATER

A Sample Container

- 1 Type container - glass. The cap liner must be teflon or solvent rinsed aluminum foil. The sample container should be rinsed with pesticide quality acetone or hexane. Allow the solvent to evaporate.

NOTE: Under no circumstances should a plastic container be used because of contamination of the sample with plasticizer and loss of organic chemicals by absorption on the container walls.

- 2 Volume - 1 liter is the minimum required.
- 3 Shipping container - special precautions must be taken to prevent breakage of liter size glass bottles filled with water. We recommend using expanded polystyrene foam shipping containers molded to fit the bottle.

- B Preservation - refrigerate to retard biological action, and reduce volatility of the constituents. The samples should be cooled by icing, packed in the polystyrene foam shippers (the foam shippers insulate the cooled sample and maintain the low temperature until received in the laboratory), and shipped to the laboratory immediately.

Many organic chemicals and pesticides are not very stable in water as indicated in the following survey.

Stability - a study of Little Miami River water spiked with 10 $\mu\text{g/l}$ of the indicated pesticides gave the following recoveries in the interval specified: BHC, heptachlor, epoxide, dieldrin, DDE, DDD, DDT,

and endrin - 100% after eight weeks, aldrin - 20% after eight weeks, heptachlor, telodrin, and endosulfan - 0% after four weeks, azodrin - 100% after eight weeks, ethion and dimethoate - 50% after eight weeks, DEF - < 5% after eight weeks, parathion 0% after eight weeks, methyl parathion, malathion, trithion, and fenthion - 0% after four weeks.

C Sampling

1 Flowing stream

- a Take a minimum of three samples in the kill area.
- b Take a control sample well above the suspected source of the pollutant.
- c Take a sample or two downstream of the kill area in case the toxic pollutant is further downstream than the area of dying fish.

2 Pond or lake

- a Take a minimum of three samples in the kill area. Samples from different depths may be required.
- b Take a control sample from the stream feeding the pond or lake, well above the suspected source of the pollutant or from another portion of the lake.

NOTE: A control sample is an absolute necessity to rule out contamination of samples or bottles during sampling or laboratory analysis.

- 3 Labeling - label the container with the sampling site, time, date and name of sampler. Indicate whether the sample is a control sample, sample from kill site or downstream of kill site.

II FISH OR OTHER BIOLOGICAL SPECIMENS

A Sample Container

- 1 Whole fish - wrap in aluminum foil and label. The foil wrapped fish can be placed in plastic bags to separate samples from different sampling sites.
- 2 Fish tissues - place in small glass jars with a teflon or foil lid liner. The jar should be solvent rinsed before use with a pesticide quality solvent such as acetone or hexane. If jars are unavailable, wrap the tissue in aluminum foil.

B Sampling

- 1 Kill area - collect about six fish from each species affected. The number is contingent on size of fish. See B.3. below. Sample two species of game fish, two of bottom feeders and two of forage fish if all species are involved in a kill.
- 2 Control samples - collect control fish of the same size and species as the affected fish. If possible, six fish of each species should be included.

NOTE: Control samples are an absolute necessity to provide a baseline for comparison of fish from the kill area. They should be collected well upstream of the suspected source of the pollutant or from a nearby body of water if upstream sampling is impossible.

The control and distressed samples should be handled in the same way concerning preservation, and shipping. This is especially important if acetylcholine esterase inhibition is to be determined.

3 Sample size

- a 3-6 inch fish are an ideal size for laboratory analysis therefore send the whole fish.
 - b Smaller fish - take enough to provide 10-50 gm/sample.
 - c Larger fish - send the whole fish if an organophosphate pesticide is the suspected cause of the kill. For kills caused by other organic chemicals, a dissected composite of gills, liver, spleen, kidney, and about 50 gm of muscle should be sent.
- 4 Condition of fish from kill area - fish taken for laboratory analysis should be in a distressed condition. Dead or rotting fish will not be analyzed.
 - 5 Preservation - fish should be placed on wet or dry ice immediately. Maximum recommended storage on wet ice is 24 hours. For longer storage the fish should be frozen. Fish should always be packed in dry ice if they are shipped.
 - 6 Method of sampling control fish - the following techniques are acceptable - seining, gill net, hook and line, and shocking.

III Sediment

A Sample container - same as for water

B Preservation - refrigerate only

C Sampling

- 1 Kill area - collect 2-3 samples. Sample near the bank or shoreline if pesticide runoff is the suspected cause of the kill. If an industry is the suspected cause, take the samples just downstream of the effluent.

- 2 Control sample - collect a sample upstream of the suspected cause of the kill.
- 3 Labeling - same as for water.

IV GENERAL CONSIDERATIONS

- A Map - a hand drawn map of the fish kill area showing sampling sites and possible source of the organic chemical is very helpful to the analyst.
- B Investigation - find out, if possible, the name of any pesticides or organic chemicals that may have caused the kill. There are approximately 900 different pesticides and a million different organic chemicals therefore any information on the possible cause of the kill is very helpful.
- C Shipping samples
 - 1 The state pollution control office or state wildlife office should be called to determine which laboratory will perform the chemical analyses.
 - 2 The laboratory should then be called to alert the proper personnel. Mr. Jim Finger, Chief, Chemical Services Branch, 404/546-3111 is the person to call for EPA, Region IV assistance.
 - 3 The samples should either be hand delivered to the laboratory or shipped Air Mail or Air Express if hand delivery is not feasible.

D SIGNIFICANCE OF ANALYTICAL DATA

The water samples taken during a fish kill can provide the most significant analytical data for possible court action. The concentrations of pesticides in the kill area can be compared to published toxicity data and conclusions can usually be reached as to the cause of the kill. The concentrations of pesticides in fish from the kill area are not as significant because there is very little if any data that shows a correlation between pesticide concentration levels in tissue and lethal dose. The data does help to support the water concentration data as it is usually considerably higher in distressed fish than in control fish.

There is a direct correlation between acetylcholine esterase inhibition and exposure to organophosphorus pesticides so this is excellent court evidence for proving the cause of a fish kill.

The only value in analyzing sediment samples in a fish kill investigation is to pinpoint the point of entry of the toxic chemical in cases such as runoff from a field; where the operator of a spray rig may have rinsed out his tank; or if an industry had a spill. Again there would be no correlation between concentrations of toxicant in the sediment and lethal dose to fish.

Descriptors: Sampling, Organic Analysis, Chemical Analysis, Pesticides

METHOD FOR ORGANOCHLORINE PESTICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

- A This method covers the determination of various organochlorine pesticides, including some pesticidal degradation products and related compounds in industrial effluents. Such compounds are composed of carbon, hydrogen, and chlorine, but may also contain oxygen, sulfur, phosphorus, nitrogen or other halogens.
- B The following compounds may be determined individually by this method with a sensitivity of 1 µg/liter: BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, Captan, DDE, DDD, DDT, methoxychlor, endosulfan, dichloran, mirex, pentachloro-nitrobenzene and trifluralin. Under favorable circumstances, Strobane, toxaphene, chlordane (tech.) and others may also be determined. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.
- C When organochlorine pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

II SUMMARY

- A The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and/or the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides,

through use of column chromatography and liquid-liquid partition, methods for elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, micro-coulometric or electrolytic conductivity gas chromatography. Results are reported in micrograms per liter.

- B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

- A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification solvents by distillation in all-glass systems may be required. Refer to Part I, Sections 1.4 and 1.5. (1)
- B The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organochlorine pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organochlorine pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

C Polychlorinated Biphenyls (PCB's) - Special attention is called to industrial plasticizers and hydraulic fluids such as the PCB's which are a potential source of interference in pesticide analysis. The presence of PCB's is indicated by a large number of partially resolved or unresolved peaks which may occur throughout the entire chromatogram. Particularly severe PCB interference will require special separation procedures.^(2,3)

D Phthalate Esters - These compounds, widely used as plasticizers, respond to the electron capture detector and are a source of interference in the determination of organochlorine pesticides using this detector. Water leaches these materials from plastics, such as polyethylene bottles and tygon tubing. The presence of phthalate esters is implicated in samples that respond to electron capture but not to the microcoulometric or electrolytic conductivity halogen detectors or to the flame photometric detector.

E Organophosphorus Pesticides - A number of organophosphorus pesticides, such as those containing a nitro group, e.g., parathion, also respond to the electron capture detector and may interfere with the determination of the organochlorine pesticides. Such compounds can be identified by their response to the flame photometric detector.⁽⁴⁾

IV. APPARATUS AND MATERIALS

A Gas Chromatograph - Equipped with glass lined injection port.

B Detector Options:

- 1 Electron Capture - Radioactive (tritium or nickel 63)
- 2 Microcoulometric Titration
- 3 Electrolytic Conductivity

C Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

D Gas Chromatographic Column Materials:

- 1 Tubing - Pyrex (180 cm long x 4 mm ID)
- 2 Glass Wool - Silanized
- 3 Solid Support - Gas-Chrom Q (100-120 mesh)
- 4 Liquid Phases - Expressed as weight percent coated on solid support.
 - a OV-1, 3%
 - b OV-210, 5%
 - c OV-17, 1.5% plus QF-1, 1.95%
 - d QF-1, 6% plus SE-30, 4%

E Kuderna-Danish (K-D) Glassware (Kontes)

- 1 Snyder Column - three ball (macro) and two ball (micro)
- 2 Evaporative Flasks - 500 ml
- 3 Receiver Ampuls - 10 ml, graduated
- 4 Ampul Stoppers

F Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

H Micro Syringes - 10, 25, 50 and 100 μ l

I Separatory Funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

- J Blender - High speed, glass or stainless steel cup.
- K Graduated cylinders - 100, 250 and 1000 ml.
- L Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 C in foil-covered glass container. Determine lauric-acid value (See Appendix I).

V REAGENTS, SOLVENTS, AND STANDARDS

- A Ferrous Sulfate - (ACS) 30% solution in distilled water.
- B Potassium Iodide - (ACS) 10% solution in distilled water.
- C Sodium Chloride - (ACS) Saturated solution in distilled water (pre-rinse NaCl with hexane).
- D Sodium Hydroxide - (ACS) 10 N in distilled water.
- E Sodium Sulfate - (ASC) Granular, anhydrous (conditioned @ 400 C for 4 hours).
- F Sulfuric Acid - (ASC) Mix equal volumes of concentration H_2SO_4 with distilled water.
- G Diethyl Ether - Nanograde, redistilled in glass, if necessary.

- 1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

- 2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. **CAUTION:** Reaction may be vigorous if the solvent contains a high concentration of peroxides.

- 3 Distill deperoxidized ether in glass and add 2% ethanol.

- H Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - nanograde, redistill in glass if necessary.

- I Pesticide Standards - Reference grade.

VI CALIBRATION

- A Gas chromatographic operating conditions are considered acceptable if the response to dicapthon is at least 50% of full scale when ≤ 0.05 ng is injected for electron capture detection and ≥ 100 ng is injected for micro-coulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.
- B Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.
- C The elution order and retention ratios of various organochlorine pesticides are provided in Table 1, as a guide.

VII QUALITY CONTROL

- A Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended. (5)

- B Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

VIII SAMPLE PREPARATION

- A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.
- B For a sensitivity requirement of $\mu\text{g/l}$, when using microcoulometric or electrolytic conductivity methods for detection, 100 ml or more of sample will be required for analysis. If interferences pose no problem, the sensitivity of the electron capture detector should permit as little as 50 ml of sample to be used. Background information on the extent and nature of interferences will assist the analyst in choosing the required sample size and preferred detector.
- C Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

IX EXTRACTION

- A Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.
- B Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pour the organic layer into a 100 ml beaker and then pass it through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of

solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

- C Concentrate the extract in the K-D evaporator on a hot water bath.

- D Analyze by gas chromatography unless a need for cleanup is indicated (See Section 10).

X CLEAN-UP AND SEPARATION PROCEDURES

- A Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

- B Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides. Of the pesticides listed in Scope (1.2) only mirex is not efficiently recovered.

- 1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.
- 2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with

two 100 ml portions of hexane by vigorously shaking about 15 seconds.

- 3 Combine the hexane extract in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.

- 4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

- 5 Analyze by gas chromatography unless a need for further cleanup is indicated.

C Florisil Column Adsorption Chromatography

- 1 Adjust the sample extract volume to 10 ml.
- 2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.
- 3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition 10-4).

Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

- 4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

- 5 Analyze by gas chromatography.

- C Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

6% Eluate

Aldrin	DDT	Pentachloro-
BHC	Heptachlor	nitrobenzene
Chlordane	Heptachlor Epoxide	Strobane
DDD	Lindane	Toxaphene
DDE	Methoxychlor	Trifluralin
	Mirex	PCB's

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

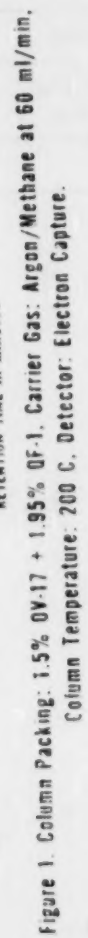
Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual.⁽⁶⁾

Table 1

RETENTION RATIOS OF VARIOUS ORGANOCHLORINE PESTICIDES RELATIVE TO ALDRIN

Liquid Phase ¹	1.5% OV-17 + 1.95% QF-1	5% OV-210	3% OV-1	6% QF-1 + 4% SE-30
Column Temp.	200 C	180 C	180 C	200 C
Argon/Methane Carrier Flow	60 ml/min	70 ml/min	70 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Trifluralin	0.39	1.11	0.33	0.57
α -BHC	0.54	0.64	0.35	0.49
PCNB	0.68	0.85	0.49	0.63
Lindane	0.69	0.81	0.44	0.60
Dichloran	0.77	1.29	0.49	0.70
Heptachlor	0.82	0.87	0.78	0.83
Aldrin	1.00	1.00	1.00	1.00
Heptachlor Epoxide	1.54	1.93	1.28	1.43
Endosulfan I	1.95	2.48	1.62	1.79
p, p'-DDE	2.23	2.10	2.00	1.82
Dieldrin	2.40	3.00	1.93	2.12
Captan	2.59	4.09	1.22	1.94
Endrin	2.93	3.56	2.18	2.42
o, p'-DDT	3.16	2.70	2.69	2.39
p, p'-DDD	3.48	3.75	2.61	2.55
Endosulfan II	3.59	4.59	2.25	2.72
p, p'-DDT	4.18	4.07	3.50	3.12
Mirex	6.1	3.78	6.6	4.79
Methoxychlor	7.6	6.5	5.7	4.60
Aldrin (Min absolute)	3.5	2.6	4.0	5.6

¹All columns glass, 180 cm x 4 mm ID, solid support Gas-Chrom Q (100/120 mesh)



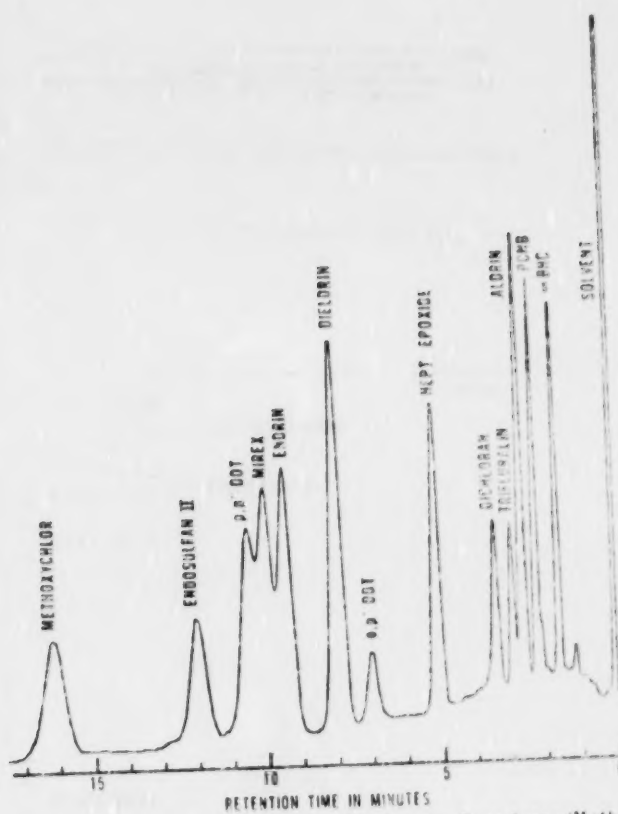


Figure 2. Column Packing: 5% GV-210. Carrier Gas: Argon/Methane at 70 ml/min. Column Temperature: 180 C. Detector: Electron Capture.

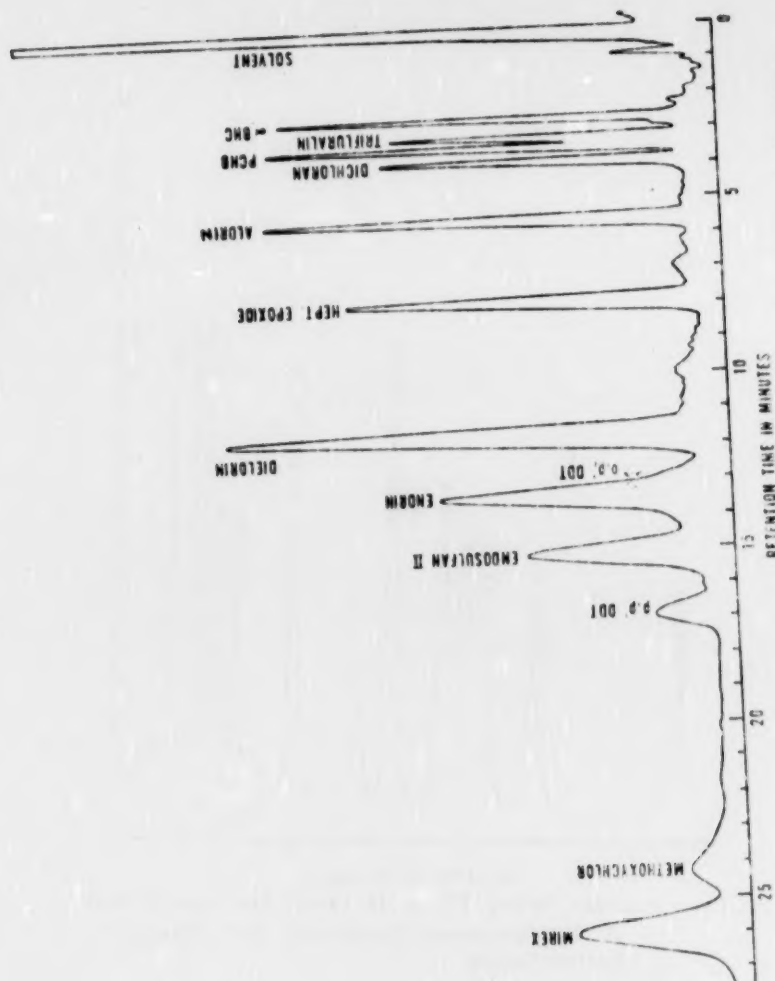


Figure 3. Column Packing: 6% QF-1 + 4% SE-30. Carrier Gas: Argon/Methane at 60 ml/min.
Column Temperature: 200 C. Detector: Electron Capture.

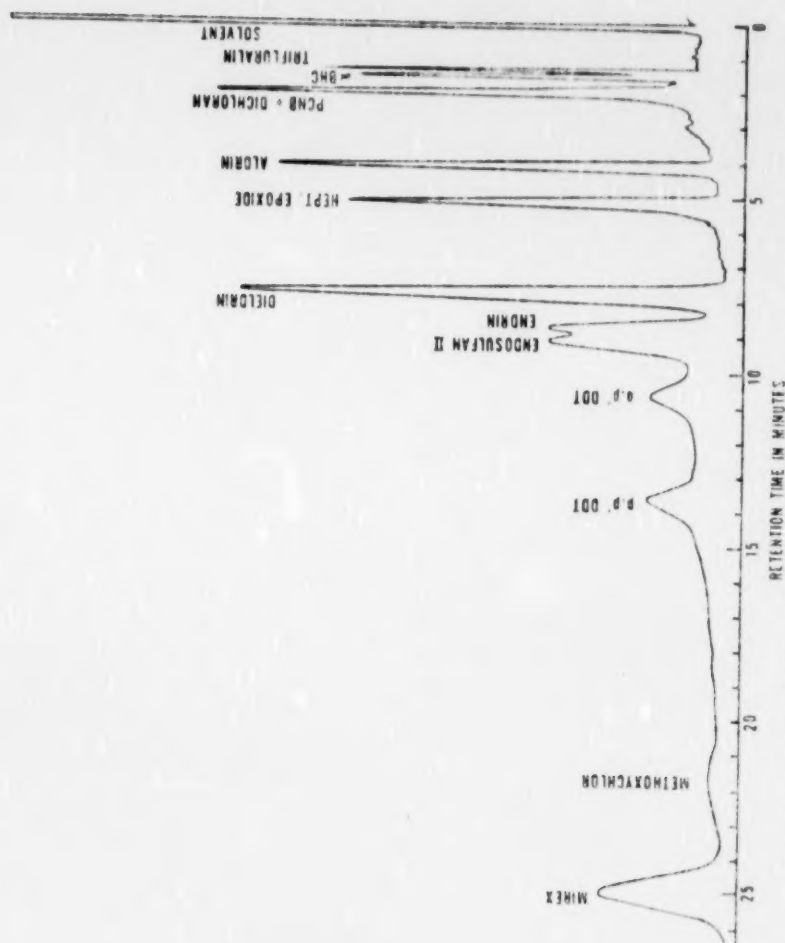


Figure 4. Column Packing: 3% OV 1. Carrier Gas: Argon/Methane at 70 ml/min.
Column Temperature: 180°C. Detector: Electron Capture.

APPENDIX

30-13

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XIII STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution. (6), (8) An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

- 1 Buret. - 25 ml with 1/10 ml graduations.
- 2 Erlenmeyer flasks. - 125 ml narrow mouth and 25 ml, glass stoppered.
- 3 Pipet. - 10 and 20 ml transfer.
- 4 Volumetric flasks. - 500 ml.

C Reagents and Solvents

- 1 Alcohol, ethyl. - USP or absolute, neutralized to phenolphthalein.
- 2 Hexane. - Distilled from all glass apparatus.
- 3 Lauric acid. - Purified, CP.
- 4 Lauric acid solution. - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 5 Phenolphthalein Indicator. - Dissolve 1 g in alcohol and dilute to 100 ml.
- 6 Sodium hydroxide. - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask.

Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

- 1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.
- 2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

- 1 Calculate amount of lauric acid adsorbed on Florisil as follows:

$$\text{Lauric Acid value} = \frac{\text{mg lauric acid/g Florisil}}{200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05\text{N NaOH})}$$
- 2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

F Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 30% eluate and the others in the 6% eluate.

METHOD FOR ORGANOPHOSPHORUS PESTICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

- A This method covers the determination of various organophosphorus pesticides and may be extended to pesticidal degradation products and related compounds. Such compounds are composed of carbon, hydrogen, and phosphorus, but may also contain sulfur, oxygen, halogen or nitrogen.
- B The following compounds may be determined individually by this method with a sensitivity of 1 $\mu\text{g/l}$: Disyston, Diazinon, malathion, Methyl Parathion, Parathion, demeton, and Guthion. Under favorable circumstances other organophosphorus pesticides may also be determined. However, the usefulness of the method for other specific compounds must be demonstrated by the analyst before applying it to sample analysis.
- C When organophosphorus pesticides exist as complex mixtures, the individual compounds may be difficult to distinguish. High, low, or otherwise unreliable results may be obtained through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method are intended to minimize the occurrence of such interferences.

II SUMMARY

- A The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and the complexity of the pesticide mixtures found. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of column chromatography and liquid-liquid partition, methods for the elimination of non-pesticide interferences and the pre-separation of pesticide mixtures.

Identification is made by selective gas Chromatographic separation and may be corroborated through the use of two or more unlike columns. Detection and measurement are best accomplished by flame photometric gas chromatography using a phosphorus specific filter. The electron capture detector, though non-specific, may also be used for those compounds to which it responds. Results are reported in micrograms per liter.

- B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

- A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part I, Sections 1.4, 1.5.⁽¹⁾
- B The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of organophosphorus pesticides. Sample clean-up procedures are generally required and may result in the loss of certain organophosphorus pesticides. Therefore, great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

C Compounds such as organochlorine pesticides, polychlorinated biphenyls and phthalate esters interfere with the analysis of organophosphorus pesticides by electron capture gas chromatography. When encountered these interferences are overcome by the use of the phosphorus specific flame photometric detector. If such a detector is not available, these interferences may be removed from the sample by using the clean-up procedures described in the EPA Methods for those compounds. (2, 3)

D Elemental sulfur will interfere with the determination of organophosphorus pesticides by flame photometric and electron capture gas chromatography. The elimination of elemental sulfur as an interference is described in Section 10.5, Clean-up and Separation Procedures.

IV APPARATUS AND MATERIALS

A Gas Chromatograph - Equipped with glass lined injection port.

B Detector Options:

- 1 Flame Photometric - 526 m μ phosphorus filter.
- 2 Electron Capture - Radioactive (tritium or nickel-63)

C Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

D Gas Chromatographic Column Materials:

- 1 Tubing - Pyrex (180 cm long x 4 mm ID)
- 2 Glass Wool - Silanized
- 3 Solid Support - Gas-Chrom Q (100-120 mesh)

4 Liquid Phases - Expressed as weight percent coated on solid support.

- a OV-1, 3%
- b OV-210, 5%
- c OV-17, 1.5% plus QF-1, 1.95%
- d QF-1, 6% plus SE-30, 4%

E Kuderna-Danish (K-D) Glassware (Kontes)

- 1 Snyder Column - three ball (macro) and two ball (micro)
- 2 Evaporative Flasks - 500 ml
- 3 Receiver Ampuls - 10 ml, graduated
- 4 Ampul Stoppers

F Chromatographic Column - Chromaflex (400 mm x 19 mm ID) with coarse fritted plate and Teflon stopcock on bottom; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column-Pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

H Micro Syringes - 10, 25, 50 and 100 μ l

I Separatory Funnels - 125 ml, 1000 ml and 2000 ml with Teflon stopcock.

J Micro-pipets - disposable (140 mm long x 5 mm ID)

K Blender - High speed, glass or stainless steel cup.

L Graduated cylinders - 100, 250 and 1000 ml.

M Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 C in foil-covered glass container. Determine lauric acid value (See Appendix I).

- N Alumina - Woelm, neutral; deactivate by pipeting 1 ml of distilled water into 125 ml ground glass-stoppered Erlenmeyer flask. Rotate flask to distribute water over surface of glass. Immediately add 19.0 g fresh alumina through small powder funnel. Shake flask containing mixture for two hours on a mechanical shaker.⁽⁴⁾

V REAGENTS, SOLVENTS, AND STANDARDS

- A Ferrous Sulfate - (ACS) 30% solution in distilled water.
 - B Potassium Iodide - (ACS) 10% solution in distilled water.
 - C Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.
 - D Sodium Hydroxide - (ACS) 10 N in distilled water.
 - E Sodium Sulfate - (ACS) Granular, anhydrous.
 - F Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.
 - G Diethyl Ether - Nanograde, redistilled in glass, if necessary.
- 1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.
 - 2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

- 3 Distill deperoxidized ether in glass and add 2% ethanol.

- H Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - Nanograde, redistill in glass if necessary.

- I Pesticide Standards - Reference Grade.

VI CALIBRATION

- A Gas chromatographic operating conditions are considered acceptable if the response to dicapthion is at least 50% of full scale when ≥ 1.5 ng is injected for flame photometric detection and ≥ 0.06 ng is injected for electron capture detection. For all quantitative measurements the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.
- B Standards are injected frequently as a check on the stability of operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 1, 2, 3 and 4 and provide reference operating conditions for the four recommended columns.
- C The elution order and retention ratios of various organophosphorus pesticides are provided in Table 1, as a guide.

VII QUALITY CONTROL

- A Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.⁽⁵⁾
- B Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

VIII SAMPLE PREPARATION

- A Blend the sample, if suspended matter is present, and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.
- B For a sensitivity requirement of $1\mu\text{g/l}$, when using flame photometric or electron capture for detection, 100 ml or more of sample will be required for analysis.
- C Quantitatively transfer the proper aliquot of sample into a two-liter separatory funnel and dilute to one liter with distilled water.

IX EXTRACTION

- A Add 60 ml of 15% methylene chloride in hexane (v:v) to the sample in the separatory funnel and shake vigorously for two minutes.
- B Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pour the organic layer into a 100 ml beaker and then pass it through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.
- C Concentrate the extract in the K-D evaporator on a hot water bath.
- D Analyze by gas chromatography unless a need for cleanup is indicated. (See Section 10).

X CLEAN-UP AND SEPARATION PROCEDURES

- A Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether clean-up is required. When these interfere with measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.
- B Acetonitrile Partition - This procedure is used to isolate fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for specific pesticides.
 - 1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.
 - 2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-45 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.
 - 3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.
 - 4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.

- 5 Analyze by gas chromatography unless a need for further clean-up is indicated.

C Florisil Column Adsorption Chromatography

- 1 Adjust the sample extract volume to 10 ml.
- 2 Place a charge of activated Florisil (weight determined by lauric-acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.
- 3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to four eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition, 10.4) Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ether.
- 4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.
- 5 Analyze by gas chromatography.

- D Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric-acid value, the pesticides will be separated into the eluates indicated above:

6% Eluate

Dameton
Disyston

15% Eluate

Diazinon
Malathion (trace)
Methyl Parathion

50% Eluate

Malathion
Guthion (20%)

100% Eluate

Guthion (80%)

For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual. (6)

- E Removal of Sulfur - If elemental sulfur interferes with the gas chromatographic analysis, it can be removed by the use of an alumina microcolumn.
- 1 Adjust the sample extract volume to 0.5 ml in a K-D apparatus, using a two-ball Snyder microcolumn.
 - 2 Plug a disposable pipet with a small quantity of glass wool. Add enough alumina to produce a 3 cm column after settling. Top the alumina with a 0.5 cm layer of anhydrous sodium sulfate.
 - 3 Quantitatively transfer the concentrated extract to the alumina microcolumn using a 100 μ l syringe. Rinse the ampul with 200 μ l of hexane and add to the microcolumn.
 - 4 Elute the microcolumn with 3 ml of hexane and discard the first eluate which contains the elemental sulfur.
 - 5 Next elute the column with 5 ml of 10% hexane in methylene chloride. Collect the eluate in a 10 ml graduated ampul.
 - 6 Analyze by gas chromatography.

NOTE: If the electron capture detector is to be used methylene chloride must be removed. To do this, attach the ampul to a K-D apparatus (500 ml flask and 3-ball Snyder column) and concentrate to about 0.5 ml. Adjust volume as required prior to analysis.

XI CALCULATION OF RESULTS

- A Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2. (1)

$$1 \text{ Micrograms/liter} = \frac{(A) (B) (V_t)}{(V_i) (V_s)}$$

A = $\frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (μl)

V_t = Volume of total extract (μl)

V_s = Volume of water extracted (ml)

XII REPORTING RESULTS

- A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

Table 1

RETENTION TIMES OF SOME ORGANOPHOSPHORUS PESTICIDES
RELATIVE TO PARATHION

Liquid Phase ¹	1.5% OV-17 + 1.95% QF-1	6% QF-1 + 4% SE-30	3% OV-210	7% OV-1
Column Temp.	215 C	215 C	200 C	200 C
Nitrogen Carrier Flow	70 ml/min	70 ml/min	60 ml/min	60 ml/min
Pesticide	RR	RR	RR	RR
Naled	with solvent	0.11 0.15	with solvent	with solvent
DDVP	0.16	0.16	0.13	0.29
Phosdrin	0.26	0.24	0.23	0.36
Demeton ²	0.46	0.26 0.43	0.20 0.38	0.74
Thimet	0.35	0.35	0.23	0.47
Diazinon	0.40	0.38	0.25	0.59
Disulfoton	0.46	0.45	0.31	0.62
Dimethoate	0.65	0.57	0.58	0.72
Ronnel	0.65	0.60	0.43	0.83
Merphos	0.69	0.67	0.34	1.23
Malathion	0.86	0.78	0.73	0.92
Methyl Parathion	0.82	0.80	0.81	0.79
Parathion	1.00	1.00	1.00	1.00
Phosphamidon ²	0.98	1.06	1.30	0.87
DEF	1.25	1.12	0.78	1.78
Ethion	2.04	1.58	2.27	2.26
Trithion	2.21	1.66	1.18	2.57
EPN	4.23	3.32	3.37	3.84
Guthion	6.65	4.15	4.44	4.68
Parathion (min absolute)	4.5	6.5	5.7	3.1

¹All columns glass, 180 cm × 4 mm ID, solid support Gas-Chrom Q, 100/120 mesh.²Anomalous, multipeak response often encountered.

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- 4 Law, L. M. and Goerlitz, D. F., "Micro-column Chromatographic Clean-up for the Analysis of Pesticides in Water," Journal of the Association of Official Analytical Chemists, 53, 1276 (1970).
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- 10 Steere, N. V., editor, "Handbook of Laboratory Safety," Chemical Rubber Company, 18901 Cranwood Parkway, Cleveland, Ohio 44128, 1971, pp. 250-254.

Descriptors: Organic Compounds Pesticides, Chlorinated Hydrocarbon Pesticides, Organophosphorus Pesticides, Techniques Analytical, Chromatography, Gas Chromatography

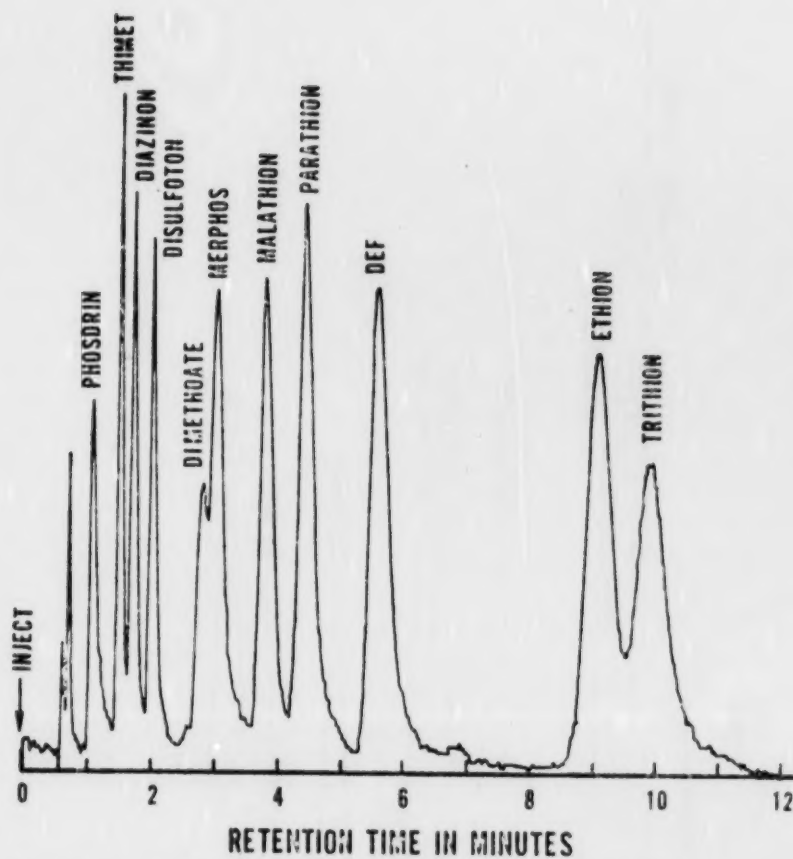


Figure 1. Column Packing: 1.5% OV-17 + 1.95 % QF-1,
Carrier Gas: Nitrogen at 70 ml/min, Column Temperature: 215 C,
Detector: Flame Photometric (Phosphorus).

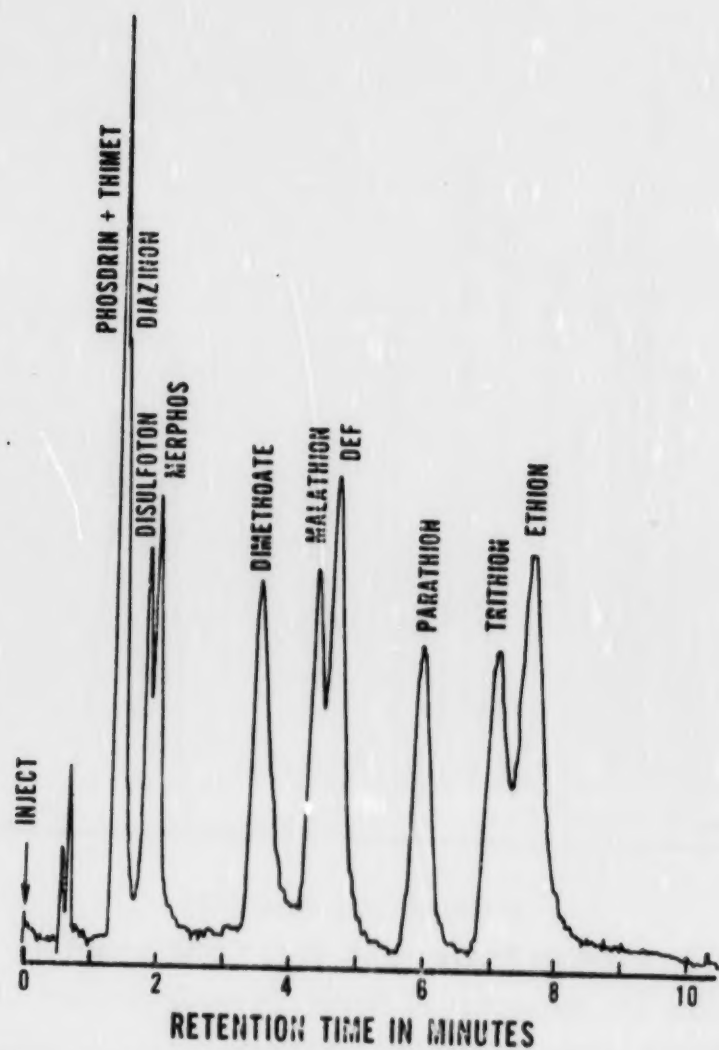


Figure 2. Column Packing: 5% OV-210, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Flame Photometric (Phosphorus).

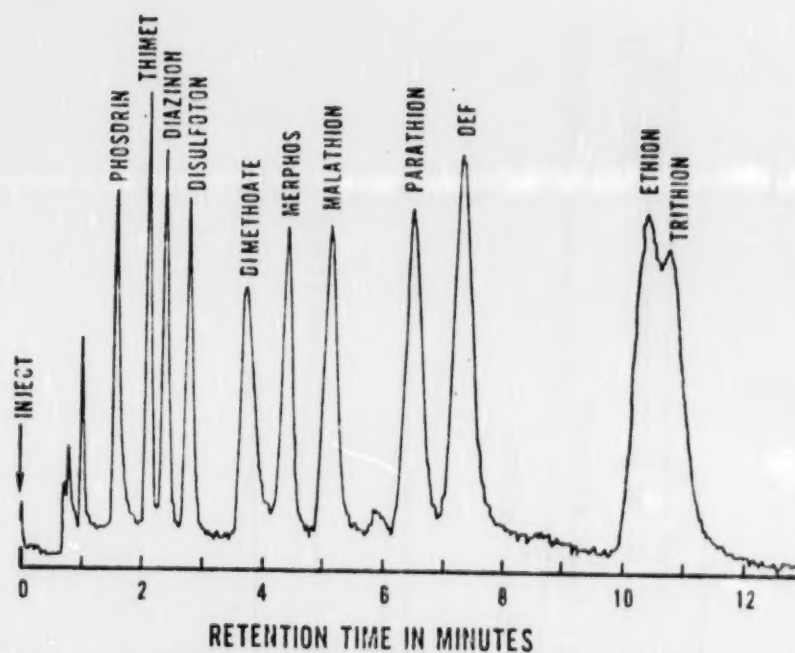


Figure 3. Column Packing: 6% QF-1 + 4% SE-30, Carrier Gas: Nitrogen at 70 ml/min, Column Temperature: 215 C, Detector: Flame Photometric (Phosphorus).

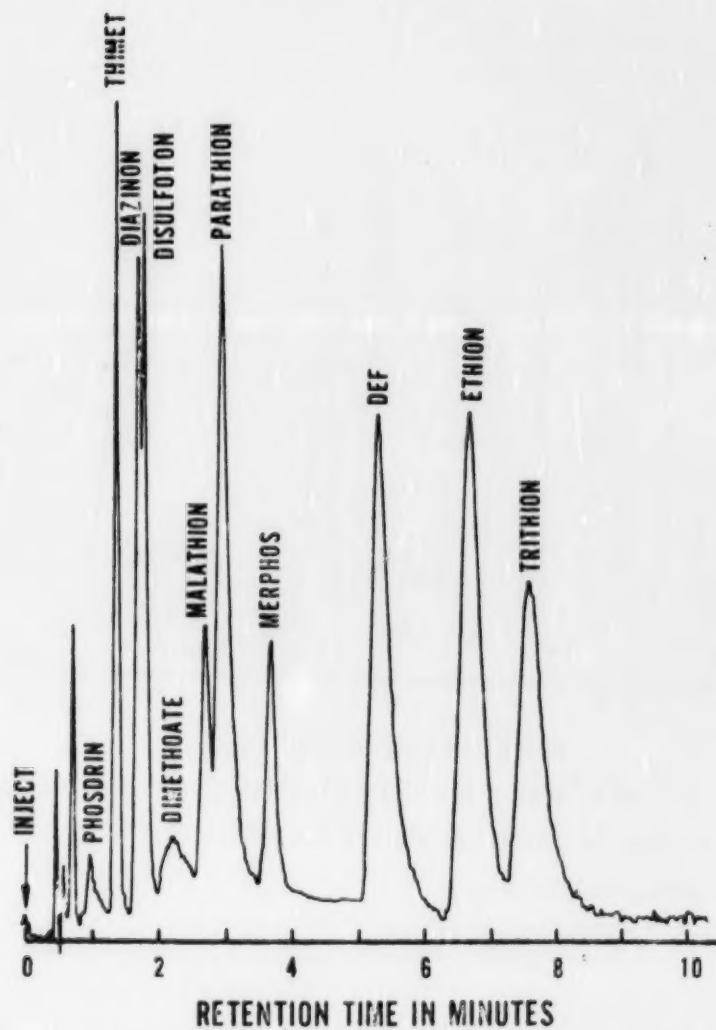


Figure 4. Column Packing: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Flame Photometric (Phosphorus).

APPENDIX

31-13

XIII STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution. (6), (8) An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

- 1 Buret. - 25 ml with 1/10 ml graduations.
- 2 Erlenmeyer flasks. - 125 ml narrow mouth and 25 ml, glass stoppered.
- 3 Pipet. - 10 and 20 ml transfer.
- 4 Volumetric flasks. - 500 ml.

C Reagents and Solvents

- 1 Alcohol, ethyl. - USP or absolute, neutralized to phenolphthalein.
- 2 Hexane. - Distilled from all glass apparatus.
- 3 Lauric acid. - Purified, CP.
- 4 Lauric acid solution. - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 5 Phenolphthalein Indicator. - Dissolve 1 g in alcohol and dilute to 100 ml.
- 6 Sodium hydroxide. - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol

and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

- 1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.
- 2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

- 1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = 200 - (ml required for titration X mg lauric acid/ml 0.05N NaOH).
- 2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

F Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

METHOD FOR POLYCHLORINATED BIPHENYLS (PCB'S) IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

- A This method covers the determination of certain polychlorinated biphenyl (PCB) mixtures including: Aroclors 1221, 1232, 1242, 1248, 1254, 1260 and 1016.
- B The method is an extension of the method for organochlorine pesticides in industrial effluents.⁽¹⁾ It is designed so that determination of both the PCB's and the organochlorine pesticides may be made on the same sample.
- C The limit of detection is approximately 1 µg/l for each Aroclor mixture.

II SUMMARY

- A The PCB's and the organochlorine pesticides are co-extracted by liquid-liquid extraction and, insofar as possible, the two classes of compounds separated from one another prior to gas chromatographic determination. A combination of the standard Florisil column cleanup procedure and a silica gel micro-column separation procedure are employed.^(2,3) Identification is made from gas chromatographic patterns obtained through the use of two or more unlike columns. Detection and measurement is accomplished using an electron capture, microcoulometric, or electrolytic conductivity detector. Techniques for confirming qualitative identification are suggested.

III INTERFERENCES

- A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms.

All of these materials must be demonstrated to be free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to ⁽⁴⁾, Part I, Section 1.4 and 1.5.

- B The interferences in industrial effluents are high and varied and pose great difficulty in obtaining accurate and precise measurement of PCB's and organochlorine pesticides. Separation and cleanup procedures are generally required to eliminate these interferences; however, such techniques may result in the loss of certain organochlorine compounds. For this reason great care should be exercised in the selection and use of methods for eliminating or minimizing interferences. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial wastes.
- C Phthalate esters, certain organophosphorus pesticides, and elemental sulfur will interfere when using electron capture for detection. These materials do not interfere when the microcoulometric or electrolytic conductivity detectors are used in the halogen mode.
- D Organochlorine pesticides and other halogenated compounds constitute interferences in the determination of PCB's. Most of these are separated by the method described below. However, certain compounds, if present in the sample, will occur with the PCB's. Included are: Sulfur, Heptachlor, aldrin, DDE, technical chlordane, mirex, and to some extent o,p'-DDT and p,p'-DDT.

IV APPARATUS AND MATERIALS

- A Gas Chromatograph - Equipped with glass lined injection port.

B Detector Options:

- 1 Electron Capture - Radioactive (tritium or nickel-63)
- 2 Microcoulometric Titration
- 3 Electrolytic Conductivity

C Recorder - Potentiometric strip chart (10 in.) compatible with detector system.

D Gas Chromatographic Column Materials:

- 1 Tubing - Pyrex (180 cm long x 4 mm ID)
- 2 Glass Wool - Silanized
- 3 Solid Support - Gas-Chrom Q (100-120 mesh)
- 4 Liquid Phases - Expressed as weight percent coated on solid support:
 - a SE-30 or OV-1, 3%
 - b OV-17, 1.5% + QF-1, 1.95%

E Kuderna-Danish (K-D) Glassware (Kontes)

- 1 Snyder Columns - three ball (macro)
- 2 Evaporate Flask - 500 ml
- 3 Receiver Ampuls - 10 ml, graduated
- 4 Ampul stoppers

F Chromatographic Column - Chroma-flex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - Pyrex (approximately 400 mm long x 20 mm ID) with a coarse fritted plate on bottom.

H Micro Column Pyrex - constructed according to Figure 1.

I Capillary pipets disposable (5-3/4 in.) with rubber bulb. (Scientific Products P5205-1).

J Low pressure regulator - 0 to 5 PSIG - with low-flow needle valve (See Figure 1, Matheson Model 70).

K Beaker - 100 ml

L Micro syringes - 10, 25, 50 and 100 μ l.

M Separatory Funnels - 125 ml, 100 ml, and 2000 ml with Teflon stopcocks.

N Graduated Cylinders - 100 ml, 250 ml and 1000 ml.

O Blender - High Speed, glass or stainless cup.

P Florisil - PR Grade (60-100 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130 in foil-covered glass container. Determine lauric-acid value (See Appendix I).

Q Silica gel - Davison code 950-08-08-226 (60/80 mesh).

R Glass Wool - Hexane extracted.

S Centrifuge Tubes - Pyrex calibrated (15 ml).

V REAGENTS, SOLVENTS AND STANDARDS

A Ferrous Sulfate - (ACS) 30% solution in distilled water.

B Potassium Iodide - (ACS) 10% solution in distilled water.

C Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

D Sodium Hydroxide - (ACS) 10 N in distilled water.

E Sodium Sulfate - (ACS) Granular, anhydrous, conditioned for 4 hours @ 400 C.

F Sulfuric Acid - (ACS) Mix equal volumes of concentrated H_2SO_4 with distilled water.

G Diethyl Ether - Nanograde, redistilled in glass, if necessary.

1 Must contain 2% alcohol and be free of peroxides by following test: to 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

3 Distill deperoxidized ether in glass and add 2% ethanol.

H n-Hexane - Pesticide quality (NOT MIXED HEXANES).

I Acetonitrile, Hexane, Methanol, Methylene Chloride, Petroleum Ether (Boiling range 30-60°C) - pesticide quality, redistill in glass if necessary.

J Standards - Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1016.

K Anti-static Solution - STATNUL, Daystrom, Inc., Weston Instrument Division, Newark, N.J. 95212.

VI CALIBRATION

Gas chromatographic operating conditions are considered acceptable when the response to dicapthion is at least 50% of full scale when $\geq .06$ ng is injected for electron capture detection and ≥ 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

B Standards are injected frequently as a check on the stability of operating conditions, detector and column. Example chromatograms are shown in Figures 3 through 8 and provide reference operating conditions.

VII QUALITY CONTROL

A Duplicate and spiked sample analyses are recommended as a quality control check. When the routine occurrence of a pollution parameter is observed, quality control charts are also recommended.⁽⁵⁾

B Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equal to that used to dilute the sample.

VIII SAMPLE PREPARATION

A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

B For a sensitivity requirement of 1 μ g/l, when using microcoulometric or electrolytic conductivity methods for detection take 1000 ml of sample for analysis. If interferences pose no problem the sensitivity of the electron capture detector should permit as little as 100 ml of sample to be used. Background information on the extent and nature of interferences will assist the

analyst in choosing the required sample size and preferred detector.

- C Quantitatively transfer the proper aliquot into a two-liter separatory funnel and dilute to one liter.

IX EXTRACTION

- A Add 60 ml of 15% methylene chloride in hexane (v/v) to the sample in the separatory funnel and shake vigorously for two minutes.
- B Allow the mixed solvent to separate from the sample, then draw the water into a one-liter Erlenmeyer flask. Pour the organic layer into a 100 ml beaker and then pass it through a column containing 3-4 inches of anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Return the water phase to the separatory funnel. Rinse the Erlenmeyer flask with a second 60 ml volume of solvent; add the solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.
- C Concentrate the extract to 6-10 ml in the K-D evaporator on a hot water bath.
- D Qualitatively analyze the sample by gas chromatography with an electron capture detector. From the response obtained decide:
- 1 If there are any organochlorine pesticides present,
 - 2 If there are any PCB's present,
 - 3 If there is a combination of a and b,
 - 4 If elemental sulfur is present,
 - 5 If the response is too complex to determine a, b, or c.
- 6 If no response, concentrate to 1.0 ml or less, as required, according to EPA Method,⁽⁴⁾ pg. 28 and repeat the analysis looking for a, b, c, d, and e. Samples containing Aroclors with a low percentage of chlorine, eg. 1221 and 1232, may require this concentration in order to achieve the detection limit of 1 µg/l. Trace quantities of PCB's are often masked by background which usually occur in the samples
- E If condition a exists, quantitatively determine the organochlorine pesticides according to (1).
- F If condition b exists, PCB's only are present, no further separation or cleanup is necessary. Quantitatively determine the PCB's according to 11. below.
- G If condition c exists, compare peaks obtained from the sample to those of standard Aroclors and make a judgment as to which Aroclors may be present. To separate the PCB's from the organochlorine pesticides, continue as outlined in 10.4.
- H If condition d exists separate the sulfur from the sample using the method outlined in (10.3) followed by the method in (10.5).
- I If condition e exists then the following macro cleanup and separation procedures (10.2 and 10.3) should be employed and, if necessary, followed by the micro separation procedures (10.4 and 10.5)

X CLEANUP AND SEPARATION PROCEDURES

- A Interferences in the form of distinct peaks and/or high background in the initial gas chromatographic analysis, as well as, the physical characteristics of the extract (color, cloudiness, viscosity) and background knowledge of the sample will indicate whether cleanup is required. When these interfere with

measurement of the pesticides, or affect column life or detector sensitivity, proceed as directed below.

B Acetonitrile Partition - This procedure is used to remove fats and oils from the sample extracts. It should be noted that not all pesticides are quantitatively recovered by this procedure. The analyst must be aware of this and demonstrate the efficiency of the partitioning for the compounds of interest.

- 1 Quantitatively transfer the previously concentrated extract to a 125 ml separatory funnel with enough hexane to bring the final volume to 15 ml. Extract the sample four times by shaking vigorously for one minute with 30 ml portions of hexane-saturated acetonitrile.
- 2 Combine and transfer the acetonitrile phases to a one-liter separatory funnel and add 650 ml of distilled water and 40 ml of saturated sodium chloride solution. Mix thoroughly for 30-35 seconds. Extract with two 100 ml portions of hexane by vigorously shaking about 15 seconds.
- 3 Combine the hexane extracts in a one-liter separatory funnel and wash with two 100 ml portions of distilled water. Discard the water layer and pour the hexane layer through a 3-4 inch anhydrous sodium sulfate column into a 500 ml K-D flask equipped with a 10 ml ampul. Rinse the separatory funnel and column with three 10 ml portions of hexane.
- 4 Concentrate the extracts to 6-10 ml in the K-D evaporator in a hot water bath.
- 5 Analyze by gas chromatography unless a need for further cleanup is indicated.

C Florisil Column Adsorption Chromatography

- 1 Adjust the sample extract volume to 10 ml.
- 2 Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.
- 3 Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and, separately, collect up to three eluates in 500 ml K-D flasks equipped with 10 ml ampuls. (See Eluate Composition below). Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated on the following page:

6% Eluate

Aldrin	DDT	Pentachloro-
BHC	Heptachlor	nitrobenzene
Chlordane	Heptachlor Epoxide	Strobane
DDD	Lindane	Toxaphene
DDE	Methoxychlor	Trifluralin
	Mirex	PCB's

15% Eluate

Endosulfan I
Endrin
Dieldrin
Dichloran
Phthalate esters

50% Eluate

Endosulfan II
Captan

Certain thiophosphate pesticides will occur in each of the above fractions as well as the 100% fraction. For additional information regarding eluate composition, refer to the FDA Pesticide Analytical Manual.(6)

- 4 Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath.

- 5 Analyze by gas chromatography.

D Silica Gel Micro-Column Separation Procedure (7)

1 Activation for Silica Gel

- a Place about 20 gm of silica gel in a 100 ml beaker. Activate at 180 C for approximately 16 hours. Transfer the silica gel to a 100 ml glass stoppered bottle. When cool, cover with about 35 ml of 0.50% diethyl ether in benzene (volume:volume). Keep bottle well sealed. If silica gel collects on the ground glass surfaces, wash off with the above solvent before re-sealing. Always maintain an excess of the mixed solvent in bottle (approximately 1/2 in. above silica gel). Silica gel can be effectively stored in

this manner for several days.

2 Preparation of the Chromatographic Column

- a Pack the lower 2 mm ID Section of the microcolumn with glass wool. Permanently mark the column 120 mm above the glass wool. Using a clean rubber bulb from a disposable pipet seal the lower end of the microcolumn. Fill the microcolumn with 0.50% ether in benzene (v/v) to the bottom of the 10/30 joint (Figure 1). Using a disposable capillary pipet, transfer several aliquots of the silica gel slurry into the microcolumn. After approximately 1 cm of silica gel collects in the bottom of the microcolumn, remove the rubber bulb seal, tap the column to insure that the silica gel settles uniformly. Carefully pack column until the silica gel reaches the 120 ± 2 mm mark. Be sure that there are no air bubbles in the column. Add about 10 mm of sodium sulfate to the top of the silica gel. Under low humidity conditions, the silica gel may coat the sides of the column and not settle properly. This can be minimized by wiping the outside of the column with an anti-static solution.

b Deactivation of the Silica Gel

- 1) Fill the microcolumn to the base of the 10/30 joint with the 0.50% ether-benzene mixture, assemble reservoir (using spring clamps) and fill with approximately 15 ml of the 0.50% ether-benzene mixture. Attach the air pressure device (using spring clamps) and adjust the elution rate to approximately 1 ml/min. with the air pressure control. Release the air pressure and detach reservoir just as the last of the solvent enters the

sodium sulfate. Fill the column with n-hexane (not mixed hexanes) to the base of the 10/30 fitting. Evaporate all residual benzene from the reservoir, assemble the reservoir section and fill with 5 ml of n-hexane. Apply air pressure and adjust the flow to 1 ml/min. (The n-hexane flows slightly faster than the benzene). Release the air pressure and remove the reservoir just as the n-hexane enters the sodium sulfate. The column is now ready for use.

- 2) Pipet a 1.0 ml aliquot of the concentrated sample extract (previously reduced to a total volume of 2.0 ml) on to the column. As the last of the sample passes into the sodium sulfate layer, rinse down the internal wall of the column twice with 0.25 ml of n-hexane. Then assemble the upper section of the column. As the last of the n-hexane rinse reaches the surface of the sodium sulfate, add enough n-hexane (volume predetermined, see 10.4.3 below) to just elute all of the PCB's present in the sample. Apply air pressure and adjust until the flow is 1 ml/min. Collect the desired volume of eluate (predetermined, see 10.4.3 below) in an accurately calibrated ampul. As the last of the n-hexane reaches the surface of the sodium sulfate, release the air pressure and change the collection ampul.
- 3) Fill the column with 0.50% diethyl ether in benzene, again apply air pressure and adjust flow to 1 ml/min. Collect the eluate until all

of the organochlorine pesticides of interest have been eluted (volume predetermined, see 10.4.3 below).

- 4) Analyze the eluates by gas chromatography.

3 Determination of Elution Volumes

- a The elution volumes for the PCB's and the pesticides depend upon a number of factors which are difficult to control. These include variation in:

- 1) Mesh size of the silica gel
- 2) Adsorption properties of the silica gel
- 3) Polar contaminants present in the eluting solvent
- 4) Polar materials present in the sample and sample solvent
- 5) The dimensions of the micro-columns

Therefore, the optimum elution volume must be experimentally determined each time a factor is changed. To determine the elution volumes, add standard mixtures of Aroclors and pesticides to the column and serially collect 1 ml elution volumes. Analyze the individual eluates by gas chromatography and determine the cut-off volume for n-hexane and for ether-benzene. Figure 2 shows the retention order of the various PCB components and of the pesticides. Using this information, prepare the mixtures required for calibration of the microcolumn.

- b In determining the volume of hexane required to elute the PCB's the sample volume (1 ml) and the volume of n-hexane used to

rinse the column wall must be considered. Thus, if it is determined that a 10.0 ml elution volume is required to elute the PCB's, the volume of hexane to be added in addition to the sample volume but including the rinse volume should be 9.5 ml.

- c Figure 2 shows that as the average chlorine content of a PCB mixture decreases the solvent volume for complete elution increases. Qualitative determination (9.4) indicates which Aroclors are present and provides the basis for selection of the ideal elution volume. This helps to minimize the quantity of organochlorine pesticides which will elute along with the low percent chlorine PCB's and insures the most efficient separations possible for accurate analysis.

- d For critical analysis where the PCB's and pesticides are not separated completely, the column should be accurately calibrated according to (10.4.3.1) to determine the percent of material of interest that elutes in each fraction. Then flush the column with an additional 15 ml of 0.50% ether in benzene followed by 5 ml of n-hexane and use this reconditioned column for the sample separation. Using this technique one can accurately predict the amount (%) of materials in each micro column fraction.

E Micro Column Separation of Sulfur, PCB's, and Pesticides

- 1 See procedure for preparation and packing micro column in PCB analysis section (10.4.1 and 10.4.2).
- 2 Microcolumn Calibration
 - a Calibrate the microcolumn for

sulfur and PCB separation by collecting 1.0 ml fractions and analyzing them by gas chromatography to determine the following:

- 1) The fraction with the first eluting PCB's (those present in 1260),
- 2) The fraction with the last eluting PCB's (those present in 1221),
- 3) The elution volume for sulfur,
- 4) The elution volume for the pesticides of interest in the 0.50% ether-benzene fraction.

From these data determine the following:

- 1) The eluting volume containing only sulfur (Fraction I),
- 2) The eluting volume containing the last of the sulfur and the early eluting PCB's (Fraction II),
- 3) The eluting volume containing the remaining PCB's (Fraction III),
- 4) The ether-benzene eluting volume containing the pesticides of interest (Fraction IV).

3 Separation Procedure

- a Carefully concentrate the 6% eluate from the florasil column to 2.0 ml in the graduated ampul on a warm water bath.
- b Place 1.0 ml (50%) of the concentrate into the microcolumn with a 1 ml pipet. Be careful not to get any sulfur crystals into the pipet.

- c Collect Fractions I and II in calibrated centrifuge tubes.

Collect Fractions III and IV in calibrated ground glass stoppered ampules.

- d Sulfur Removal⁽⁹⁾ - Add 1 to 2 drops of mercury to Fraction II stopper and place on a wrist-action shaker. A black precipitate indicates the presence of sulfur. After approximately 20 minutes the mercury may become entirely reacted or deactivated by the precipitate. The sample should be quantitatively transferred to a clean centrifuge tube and additional mercury added. When crystals are present in the sample, three treatments may be necessary to remove all the sulfur. After all the sulfur has been removed from Fraction II (check using gas chromatography) combine Fractions II and III. Adjust the volume to 10 ml and analyze gas chromatographically. Be sure no mercury is transferred to the combined Fractions II and III, since it can react with certain pesticides.

By combining Fractions II and III, if PCB's are present, it is possible to identify the Aroclor(s) present and a quantitative analysis can be performed accordingly. Fraction I can be discarded since it only contains the bulk of the sulfur. Analyze Fractions III and IV for the PCB's and pesticides. If DDT and its homologs, aldrin, heptachlor, or technical chlordane are present along with the PCB's, an additional microcolumn separation can be performed which may help to further separate the PCB's from the pesticides (See 10.4).

microcoulometric or the electrolytic detector may be employed to improve specificity for samples having higher concentrations of PCB's.

B Calculations

- 1 When a single Aroclor is present, compare quantitative Aroclor reference standard (e.g., 1242, 1260) to the unknown. Measure and sum the areas of the unknown and the reference Aroclor and calculate the result as follows:

$$\text{Microgram/liter} = \frac{[A] [B] [V_t]}{[(V_i) (V_s)]} \times [N]$$

$$A = \frac{\text{ng of Standard Injected}}{\Sigma \text{ of Standard Peak Areas}} = \frac{\text{ng}}{\text{mm}^2}$$

$$B = \Sigma \text{ of Sample Peak Areas} = (\text{mm}^2)$$

V_i = Volume of sample injected (μ l)

V_t = Volume of Extract (μ l) from which sample is injected into gas chromatograph

V_s = Volume of water sample extracted (ml)

$N = 2$ when micro column used
1 when micro column not used

Peak Area = Peak height (mm x Peak Width at 1/2 height)

- 2 For complex situations, use the calibration method described below. Small variations in components between different Aroclor batches make it necessary to obtain samples of several specific Aroclors. These reference Aroclors can be obtained from Dr. Ronald Webb, Southeast Environmental Research Laboratory, EPA, Athens, Georgia 30601. The procedure is as follows:

- a Using the OV-1 column, chromatograph a known quantity of each Aroclor reference standard. Also chromatograph a sample of p, p'-DDE. Suggested concentration of each standard is 0.1 ng/ μ l for the Aroclors and 0.02 ng/ μ l for the p, p'-DDE.

XI QUANTITATIVE DETERMINATION

- A Measure the volume of n-hexane eluate, containing the PCB's and inject 1 to 5 μ l into the gas chromatograph. If necessary, adjust the volume of the eluate to give linear response to the electron capture detector. The

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- b Determine the relative retention time (RRT) of each PCB peak in the resulting chromatograms using p,p'-DDE as 100. See Figures 3 through 5.

$$RRT = \frac{RT \times 100}{RT_{DDE}}$$

RRT = Relative Retention Time

RT = Retention time of peak of interest

RT_{DDE} = Retention time of p,p'-DDE

Retention time is measured as that distance in mm between the first appearance of the solvent peak and the maximum for the compound.

- c To calibrate the instrument for each PCB measure the area of each peak.

Area = Peak height (mm) x Peak width at 1/2 height. Using Tables 1 through 6 obtain the proper mean weight factor, then determine the response factor ng/mm².

$$ng/mm^2 = \frac{(ng_i) \left(\frac{\text{mean weight percent}}{100} \right)}{(\text{Area})}$$

ng_i = ng of Aroclor Standard Injected

Mean weight percent = obtained from Tables 1 through 6.

- d Calculate the RRT value and the area for each PCB peak in the sample chromatogram. Compare the sample chromatogram to those obtained for each reference Aroclor standard. If it is apparent that the PCB peaks present are due to only one Aroclor then calculate the concentration of each PCB using the following formula:

$$ng \text{ PCB} = ng/mm^2 \times \text{Area}$$

Where Area = Area₂(mm²) of sample peak ng/mm² = Response factor for that peak measured. Then add the nanograms of PCB's present in the injection to get the total number of nanograms of PCB's present. Use the following formula to calculate the concentration of PCB's in the sample:

$$\text{Micrograms/Liter} = \frac{[ng]}{[V_s]} \frac{[V_t]}{[V_i]} \times [N]$$

V_s = volume of water extracted (ml)

V_t = volume of extract (μl)

V_i = volume of sample injected (μl)

Σng = sum of all the PCB's in nanograms for that Aroclor identified

N = 2 when microcolumn used

N = 1 when microcolumn not used

The value can then be reported as Micrograms/Liter PCB's reported as the Aroclor. For samples containing more than one Aroclor, use Figure 9 chromatogram divisional flow chart to assign a proper response factor to each peak and also identify the "most likely" Aroclors present. Calculate the ng of each PCB isomer present and sum them according to the divisional flow chart. Using the formula above, calculate the concentration of the various Aroclors present in the sample.

XII REPORTING RESULTS

- A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, all data obtained should be reported.

Table 1

Composition of Aroclor 1221⁽⁸⁾

RRT ^a	Mean Weight Percent	Relative Std. Dev. ^b	Number of Chlorines ^c
11	31.8	15.8	1
14	19.3	9.1	1
16	10.1	9.7	2
19	2.8	9.7	2
21	20.8	9.3	2
28	5.4	13.9	2 } 85%
			3 } 15%
32	1.4	30.1	2 } 10%
			3 } 90%
[37 40]	1.7	48.8	3
			3
Total	93.3		

^aRetention time relative to p, p'-DDE= 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

^bStandard deviation of seventeen results as a percentage of the mean of the results.

^cFrom GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 2

Composition of Aroclor 1232 (8)

RRT ²	Mean Weight Percent	Relative Std. Dev. ^b	Number of Chlorines ^c
11	16.2	3.4	1
14	9.9	2.5	1
16	7.1	6.8	2
[20	17.8	2.4	2
21			2
28	9.6	3.4	2 } 40%
			3 } 60%
32	3.9	4.7	3
37	6.8	2.5	3
40	6.4	2.7	3
47	4.2	4.1	4
54	3.4	3.4	3 } 33%
			4 } 67%
58	2.6	3.7	4
70	4.6	3.1	4 } 90%
			5 } 10%
78	1.7	7.5	4
Total	94.2		

^a Retention time relative to p,p'-DDE= 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

^b Standard deviation of four results as a mean of the results.

^c From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 3

Composition of Aroclor 1242⁽⁸⁾

RRT ^a	Mean Weight Percent	Relative Std. Dev. ^b	Number of Chlorines ^c
11	1.1	35.7	1
16	2.9	4.2	2
21	11.3	3.0	2
28	11.0	5.0	2 } 25%
			3 } 75%
32	6.1	4.7	3
37	11.5	5.7	3
40	11.1	6.2	3
47	8.8	4.3	4
54	6.8	2.9	3 } 33%
			4 } 67%
58	5.6	3.3	4
70	10.3	2.8	4 } 90%
			5 } 10%
78	3.6	4.2	4
84	2.7	9.7	5
98	1.5	9.4	5
104	2.3	16.4	5
125	1.6	20.4	5 } 85%
			6 } 15%
146	1.0	19.9	5 } 75%
			6 } 25%
Total	98.5		

^aRetention time relative to p,p'-DDE= 100. Measured from first appearance of solvent.

^bStandard deviation of six results as a percentage of the mean of the results.

^cFrom GC-MS data. Peaks containing mixtures of isomers of different chlorine number are bracketed.

Table 4

Composition of Aroclor 1248⁽⁸⁾

RRT ^a	Mean Weight Percent	Relative Std. Dev. ^b	Number of Chlorines
21	1.2	23.9	2
28	5.2	3.3	3
32	3.2	3.8	3
37	8.3	3.6	3
40	8.3	3.9	3 } 85%
			4 } 15%
47	15.6	1.1	4
54	9.7	6.0	3 } 10%
			4 } 90%
58	9.3	5.8	4
70	19.0	1.4	4 } 80%
			5 } 20%
78	6.6	2.7	4
84	4.9	2.6	5
98	3.2	3.2	5
104	3.3	3.6	4 } 10%
			5 } 90%
112	1.2	6.6	5
125	2.6	5.9	5 } 90%
			6 } 10%
146	1.5	10.0	5 } 85%
			6 } 15%
Total	103.1		

^a Retention time relative to p,p'-DDE= 100. Measured from first appearance of solvent.

^b Standard deviation of six results as a percentage of the mean of the results.

^c From GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

Table 5

Composition of Aroclor 1254⁽⁸⁾

RRT ^a	Mean Weight Percent	Relative Std. Dev. ^b	Number of Chlorines ^c
47	6.2	3.7	4
54	2.9	2.6	4
58	1.4	2.8	4
70	13.2	2.7	4] 25%
			5] 75%
84	17.3	1.9	5
98	7.5	5.3	5
104	13.6	3.8	5
125	15.0	2.4	5] 70%
			6] 30%
146	10.4	2.7	5] 30%
			6] 70%
160	1.3	8.4	6
174	8.4	5.5	6
203	1.8	18.6	6
232	1.0	26.1	7
Total	100.0		

^aRetention time relative to p,p'-DDE= 100. Measured from first appearance of solvent.

^bStandard deviation of six results as a percentage of the mean of the results.

^cFrom GC-MS data. Peaks containing mixtures of isomers are bracketed.

Table 6

Composition of Aroclor 1260⁽⁸⁾

RRT ^a	Mean Weight Percent	Relative Std. Dev. ^b	Number of Chlorines ^c
70	2.7	6.3	5
84	4.7	1.6	5
[98	3.8	3.5	5 } ^d
104			6 } 60%
117	3.3	6.7	6 } 40%
125	12.3	3.3	5 } 15%
146	14.1	3.6	6 } 85%
160	4.9	2.2	6 } 50%
174	12.4	2.7	7 } 50%
203	9.3	4.0	6 } 10%
[232			7 } ^e
244	9.8	3.4	6 } 10%
280	11.0	2.4	8 } 90%
332	4.2	5.0	7
372	4.0	8.6	8
448	.6	25.3	8
528	1.5	10.2	8
Total	98.6		

^aRetention time relative to p,p'-DDE= 100. Measured from first appearance of solvent. Overlapping peaks that are quantitated as one peak are bracketed.

^bStandard deviation of six results as a mean of the results.

^cFrom GC-MS data. Peaks containing mixtures of isomers of different chlorine numbers are bracketed.

^dComposition determined at the center of peak 104.

^eComposition determined at the center of peak 232.

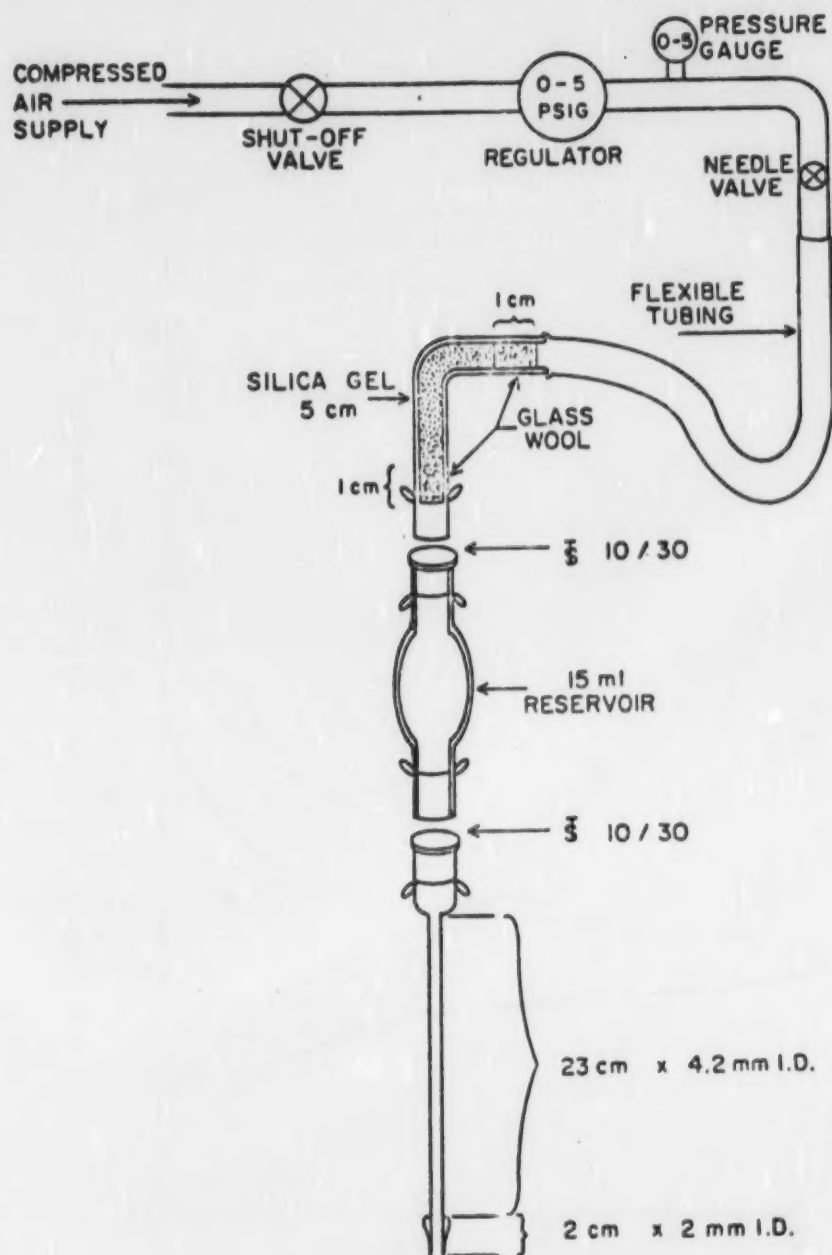


FIGURE 1. MICRO COLUMN SYSTEM

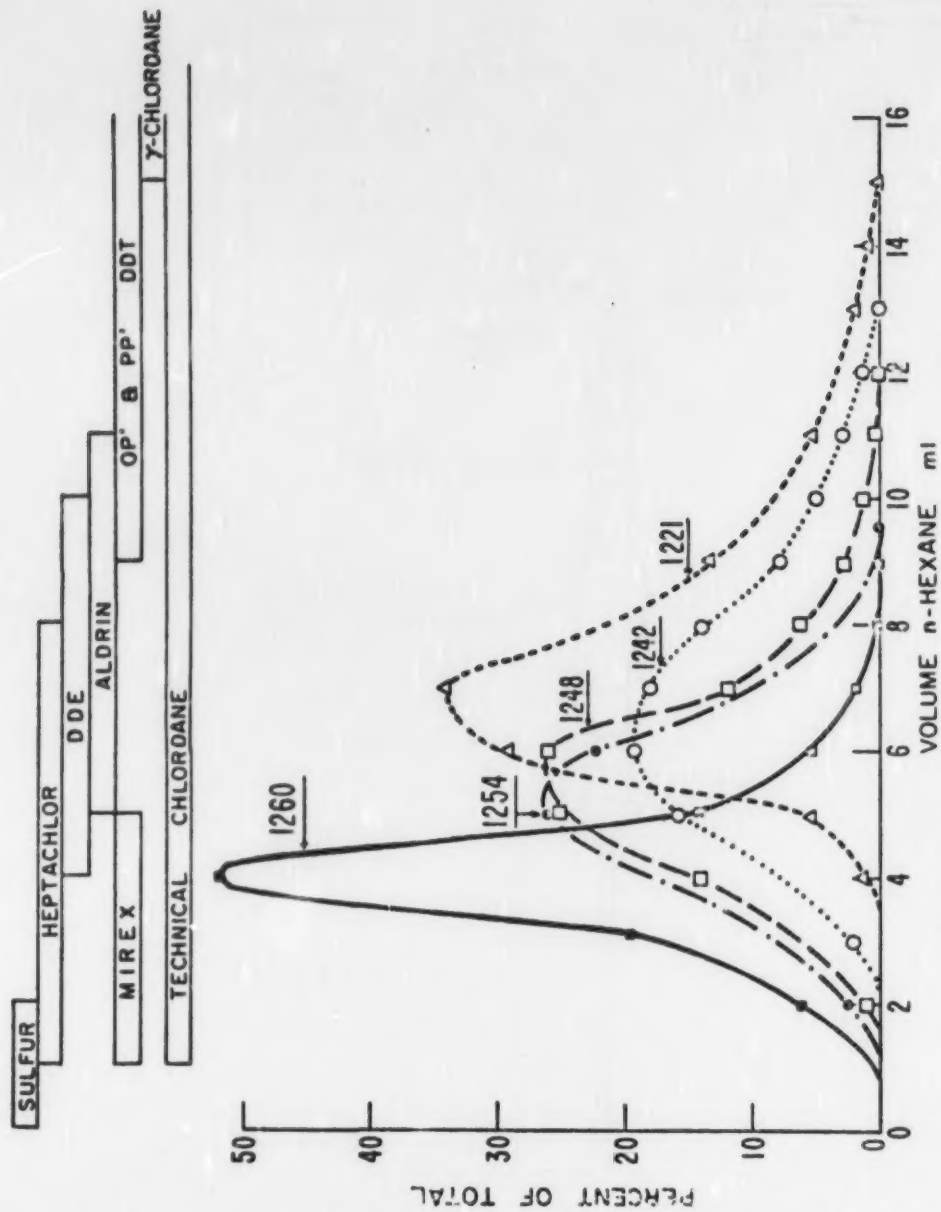


Figure 2. Aroclor Elution Patterns

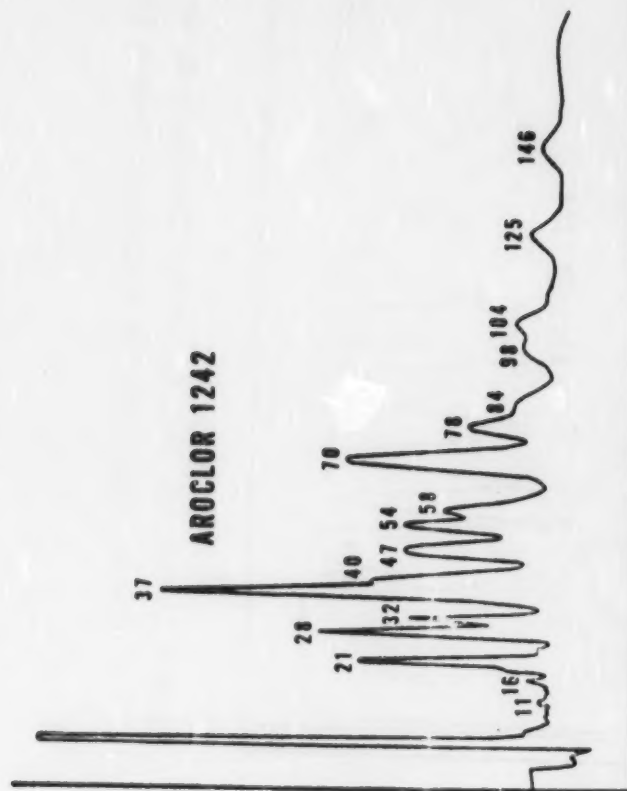


Figure 3. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C, Detector: Electron Capture

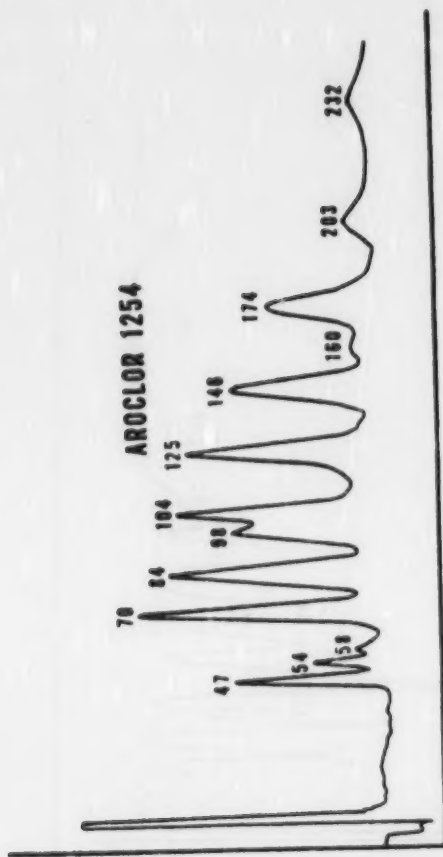


Figure 4. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C. Detector: Electron Capture.



Figure 5. Column: 3% OV-1, Carrier Gas: Nitrogen at 60 ml/min,
Column Temperature: 170 C, Detector: Electron Capture.

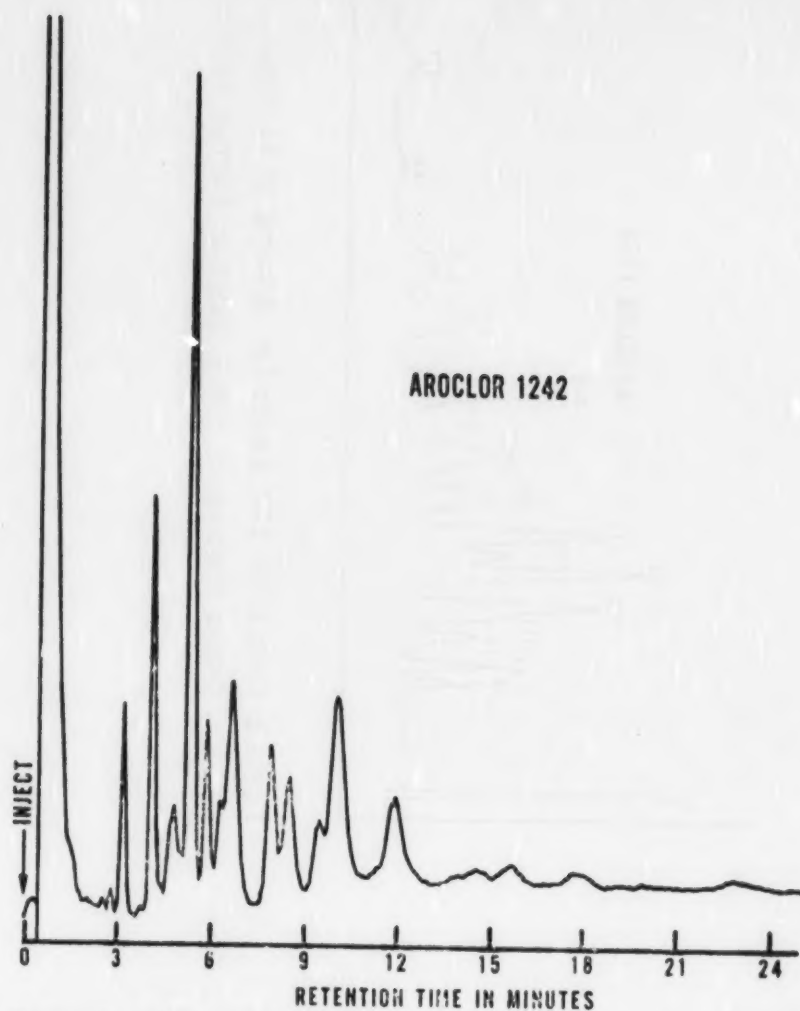


Figure 6. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

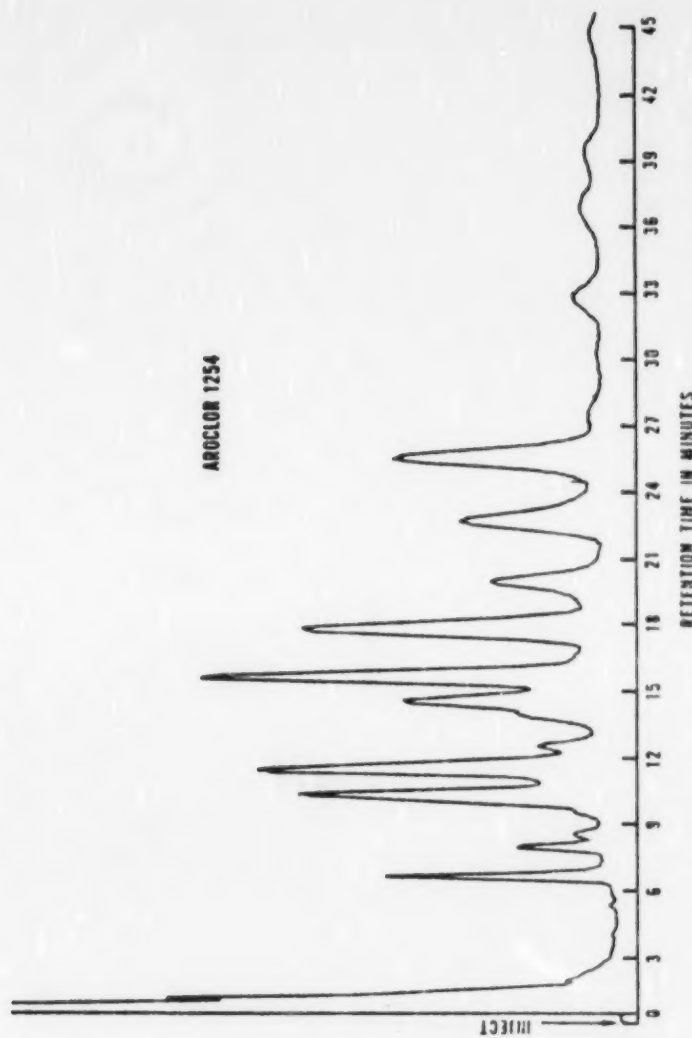


Figure 7. Column: 1.5% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200 C, Detector: Electron Capture.

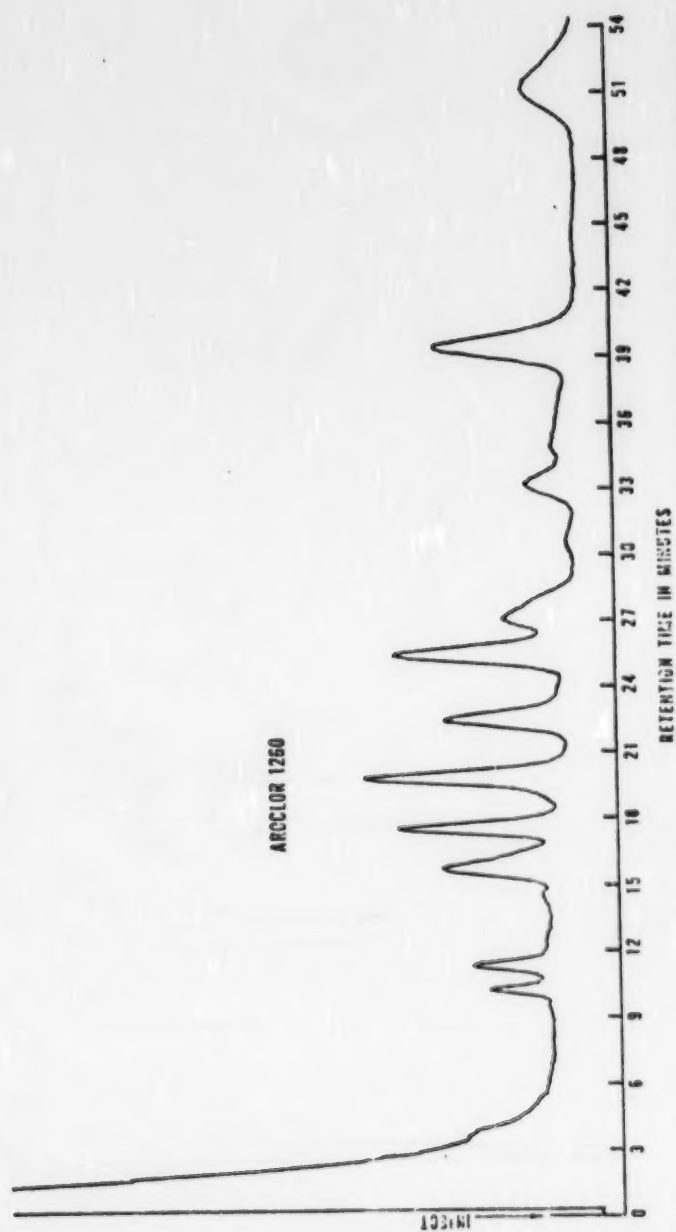


Figure 8. Column: 15% OV-17 + 1.95% QF-1, Carrier Gas: Nitrogen at 60 ml/min, Column Temperature: 200°C, Detector: Electron Capture.

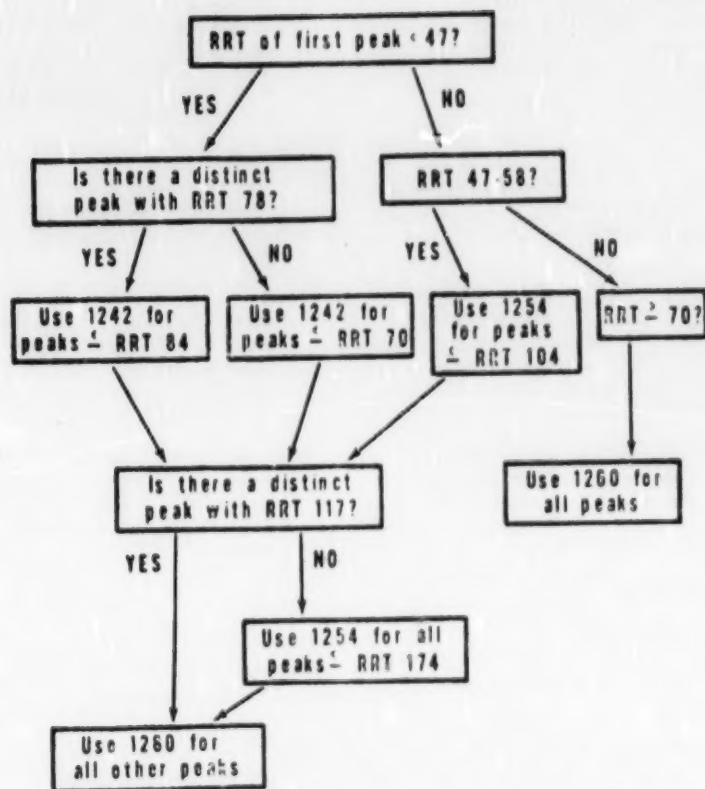


Figure 9. Chromatogram Division Flowchart (8).

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 - 2 Leoni, V. "The Separation of Fifty Pesticides and Related Compounds and Polychlorinated Biphenyls into Four Groups by Silica Gel Microcolumn Chromatography," Journal of Chromatography, **62**, 63 (1971).
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- Descriptors: Chemical Analysis, Polychlorinated Biphenyls, Chromatography, Gas Chromatography

APPENDIX I

32-27

XIII STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6), (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

- 1 Buret. - 25 ml with 1/10 ml graduations.
- 2 Erlenmeyer flasks. - 125 ml narrow mouth and 25 ml, glass stoppered.
- 3 Pipet. - 10 and 20 ml transfer.
- 4 Volumetric flasks. - 500 ml.

C Reagents and Solvents

- 1 Alcohol, ethyl. - USP or absolute, neutralized to phenolphthalein.
- 2 Hexane. - Distilled from all glass apparatus.
- 3 Lauric acid. - Purified, CP.
- 4 Lauric acid solution. - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 5 Phenolphthalein Indicator. - Dissolve 1 g in alcohol and dilute to 100 ml.
- 6 Sodium hydroxide. - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

- 1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.
- 2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

- 1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = $200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05\text{N NaOH})$.

- 2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

- F Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p, p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

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METHOD FOR TRIAZINE PESTICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

- A This method covers the determination of various symmetrical triazine pesticides.
- B The following compounds may be determined by this method with a sensitivity of 1 µg/l: ametryne, atratone, atrazine, GS-13529, GS-14254, prometone, prometryne, propazine, and simazine. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.
- C Individual triazine may be difficult to identify and quantitate in the presence of other triazines or other nitrogen-containing compounds. Provisions incorporated in this method are intended to minimize the effect of such interferences.

II SUMMARY

- A The method describes an efficient sample extraction procedure and provides, through use of column chromatography, a method for the elimination of non-pesticide interferences and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separation, and measurement is accomplished by the use of an electrolytic conductivity detector (CCD). Results are reported in micrograms per liter.
- B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

- A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be

free from interferences under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to (1) Part 1, (Sections 1.4 and 1.5).

- B The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of triazine pesticides. The use of a specific detector supported by an optional column cleanup procedure will eliminate many of these interferences.
- C Nitrogen containing compounds other than the triazines may interfere.

IV APPARATUS AND MATERIALS

- A Gas Chromatograph - Equipped with glass-lined injection port.
- B Detector - Electrolytic Conductivity
- C Recorder - Potentiometric strip chart (10 in) compatible with the detector.
- D Gas Chromatographic Column Materials:
 - 1 Tubing - Pyrex (180 cm long x 4 mm ID)
 - 2 Glass Wool - Silanized
 - 3 Solid Support - Gas-Chrom Q (100-120 mesh)
 - 4 Liquid Phase - Expressed as weight percent coated on solid support
 - a Carbowax 20M, 1%
- E Kuderna-Danish (K-D) Glassware (Kontes)
 - 1 Snyder Column - three ball (K-503000)
 - 2 Micro-Snyder Column - two ball (K-569001)
 - 3 Evaporative Flasks - 500 ml (K-570001)

4 Receiver Ampuls - 10 ml, graduated (K-570050)

5 Ampul Stoppers

F Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - Pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

H Micro Syringes - 10, 25, 50, and 100 μ l.

I Separatory Funnels - 2000 ml with Teflon stopcock.

J Blender - High speed, glass or stainless steel cup.

K Graduated Cylinders - 1000 ml.

L Florisil - PR Grade (60-80 mesh); purchase activated at 1250F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130 C in foil-covered glass container. Determine lauric-acid value (See Appendix I).

V REAGENTS, SOLVENTS AND STANDARDS

A Ferrous Sulfate - (ACS) 30% solution in distilled water.

B Potassium Iodide - (ACS) 10% solution in distilled water.

C Sodium Hydroxide - (ACS) 10 N in distilled water.

D Sodium Sulfate - (ACS) Granular, anhydrous.

E Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

F Diethyl Ether - Pesticide Quality, redistilled in glass, if necessary.

1 Must contain 2% alcohol and be free of peroxides by the following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add 1 ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

3 Distill deperoxidized ether in glass and add 2% ethanol.

G Hexane, Methanol, Methylene Chloride, Petroleum Ether (boiling range 30-60 C) - pesticide quality, redistill in glass if necessary.

H Pesticide Standards - Reference grade.

VI CALIBRATION

A Gas chromatographic operating conditions are considered optimum when an injection of ≈ 20 ng of each triazine will yield a peak at least 50% of full scale deflection with the modified Coulson detector.⁽²⁾ For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.

B Inject standards frequently as a check on the stability of operating conditions. A chromatogram of a mixture of several pesticides is shown in Figure 1 and provides reference operating conditions for the recommended column.

C The elution order and retention ratios of various triazine pesticides are provided in Table 1, as a guide.

VII QUALITY CONTROL

A Duplicate and spiked sample analyses are recommended as quality control

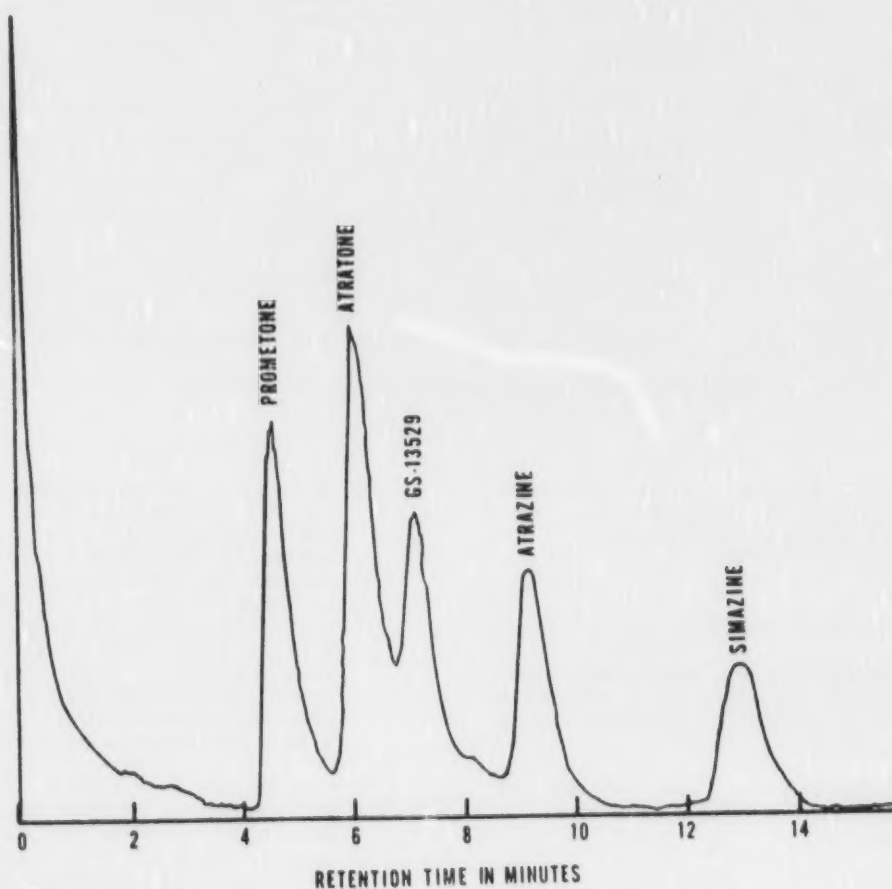


Figure 1. Column Packing: 1% Carbowax 20M on Gas-Chrom Q (100/120 mesh).
Column Temperature : 155 C, Carrier Gas: Helium at 80 ml/min,
Detector: Electrolytic Conductivity.

Table 1

RETENTION RATIOS OF VARIOUS TRIAZINE
PESTICIDES RELATIVE OF ATRAZINE

Pesticide	Retention Ratio
Prometone	0.52
Atratone	0.67
Propazine	0.71
GS-13529	0.78
GS-14254	0.88
Atrazine	1.00
Prometryne	1.10
Simazine	1.35
Ametryne	1.48

Absolute retention time of atrazine = 10.1 minutes

checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.⁽³⁾

VIII SAMPLE PREPARATION

A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.

B Quantitatively transfer a 1000 ml aliquot into a two-liter separatory funnel.

IX EXTRACTION

A Add 60 ml methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.

B Allow the solvent to separate from the sample, draw the organic layer into a 100 ml beaker, then pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Add a second 60 ml volume of solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.

C Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (10 below).

D If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by gas chromatography.

bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 ml.

F Analyze by gas chromatography.

X FLORISIL COLUMN CLEANUP

A Adjust the sample extract to 10 ml with hexane.

B Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex chromatographic column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

C Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and separately collect the eluates in 500 ml K-D flasks equipped with 10 ml ampuls. Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

D Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

15% Eluate	50% Eluate	100% Eluate
Propazine (90%)	Propazine (10%)	Atrazine
GS-13529 (30%)	GS-13529 (70%)	GS-14254
Atrazine (20%)	Atrazine (80%)	Prometon
	Ametryne	
	Prometryne	
	Simazine	

E Concentrate the eluate to 6-10 ml in the K-D evaporator in a hot water

XI CALCULATION OF RESULTS

A Determine the pesticide concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, (Section 3.4.2). (4)

$$\text{Micrograms/liter} = \frac{(A) (B) (V_t)}{(V_i) (V_s)}$$

$$A = \frac{\text{ng standard}}{\text{Standard area}}$$

B = Sample aliquot area

V_i = Volume of extract injected (μ l)

V_t = Volume of total extract (μ l)

V_s = Volume of water extracted (ml)

XII REPORTING RESULTS

A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

REFERENCES

- 1 "Methods for Organic Pesticides in Water and Wastewater", U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1971.
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- 3 "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Chapter 6, Section 6.4, U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.

- 4 Cochrane, W. P. and Wilson, B. P. "Electrolytic conductivity detection of some nitrogen-containing herbicides", Journal of Chromatography, 63, 364 (1971).

Descriptors: Chemical Analysis Pesticides, Chromatography, Gas Chromatography

APPENDIX I

33-7

XIII STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

- 1 Buret. - 25 ml with 1/10 ml graduations.
- 2 Erlenmeyer flasks. - 125 ml narrow mouth and 25 ml, glass stoppered.
- 3 Pipet. - 10 and 20 ml transfer.
- 4 Volumetric flasks. - 500 ml.

C Reagents and Solvents

- 1 Alcohol, ethyl. - USP or absolute, neutralized to phenolphthalein.
- 2 Hexane. - Distilled from all glass apparatus.
- 3 Lauric acid. - Purified, CP.
- 4 Lauric acid solution. - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 5 Phenolphthalein Indicator. - Dissolve 1 g in alcohol and dilute to 100 ml.
- 6 Sodium hydroxide. - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N).

Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

- 1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.
- 2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

- 1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = $\frac{\text{mg lauric acid/g Florisil} \times 200}{(\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05\text{N NaOH})}$
- 2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6

F Test for Proper Elution Pattern and Recovery of Pesticides:

Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

METHOD FOR O-ARYL CARBAMATE PESTICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

- A This method covers the determination of various O-aryl carbamate pesticides in industrial effluents. Such compounds are characterized by the carbamate structure with the oxygen atom attached to an aromatic ring.
- B The following compounds may be determined individually by this method with a sensitivity of 1 µg/liter: Baygon, carbaryl (Sevin), Matacil, Mesurol, and Zectran. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.
- C The method also detects phenols and can be extended to the detection of phenolic hydrolysis products of the compounds above.

II SUMMARY

- A A measured volume of water is extracted with methylene chloride. The concentrated extract is cleaned up with a Florisil column. Appropriate fractions from the column are concentrated and portions are separated by thin-layer chromatography. The carbamates are hydrolyzed on the layer and the hydrolysis products are reacted with 2,6-dibromoquinone chlorimide to yield specific colored products. Quantitative measurement is achieved by visually comparing the responses of sample extracts to the responses of standards on the same thin-layer. Identifications are confirmed by changing the pH of the layer and observing color changes of the reaction products. Results are reported in micrograms per liter.
- B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

- A Direct interferences may be encountered from phenols that may be present in the sample. These materials react with the chromogenic reagent and yield reaction products similar to those of the carbamates. In cases where phenols are suspected of interfering with a determination, a different solvent system should be used to attempt to isolate the carbamates.
- B Indirect interferences may be encountered from naturally colored materials whose presence masks the carbamate reaction.

IV APPARATUS AND MATERIALS

- A Thin layer plates - Glass plates (200 x 200 mm) coated with 0.25 mm layer of Silica Gel G (gypsum binder)
- B Spotting Template
- C Developing Chamber
- D Sprayer - 20 ml Capacity
- E Kuderna-Danish (K-D) Glassware (Kontes)
 - 1 Snyder Column - Three ball (K-503000)
 - 2 Micro-Snyder Column - Two ball (K-569001)
 - 3 Evaporative Flasks - 500 ml (K-570001)
 - 4 Receiver Ampuls - 10 ml graduated (K-570050)
 - 5 Ampul Stoppers
- F Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon

stopcock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - Pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

H Micro Syringes - 10, 25, 50 and 100 μ l.

I Separatory Funnel - 2000 ml, with Teflon stopcock.

J Blender - High speed, glass or stainless steel cup.

K Florisil - PR Grade (60-80 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130 C in foil-covered glass container. Determine lauric acid value (See Appendix I).

V REAGENTS, SOLVENTS AND STANDARDS

A Ferrous Sulfate - (ACS) 3% solution in distilled water.

B Potassium Iodide - (ACS) 10% solution in distilled water.

C Sodium Hydroxide - (ACS) 10N in distilled water.

D Sodium Sulfate - (ACS) Granular, anhydrous.

E Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

F Diethyl Ether - Nanograde, redistilled in glass, if necessary.

1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

2 Decompose ether peroxides by adding 40g of 30% ferrous sulfate solution

to each liter of solvent. **CAUTION:** Reaction may be vigorous if the solvent contains a high concentration of peroxides

3 Distill deperoxidized ether in glass and add 2% ethanol.

G Hexane, Methanol, Methylene Chloride, Petroleum Ether - Nanograde, redistill in glass if necessary.

H Pesticide Standards - Reference grade.

1 TLC standards - 0.100 μ g/ μ l in chloroform.

I Chromogenic agent - Dissolve 0.2 g 2,6-dibromoquinone chlorimide in 20 ml chloroform.

J Buffer solution - 0.1 N sodium borate in water.

VI CALIBRATION

A To insure even solvent travel up the layer, the tank used for layer development must be thoroughly saturated with developing solvent before it is used. This may be achieved by lining the inner walls of the tank with chromatography paper and introducing the solvent 1-2 hours before use.

B Samples and standards should be introduced to the layer using a syringe, micropipet or other suitable device that permits all the spots to be about the same size and as small as possible. An air stream directed on the layer during spotting will speed solvent evaporation and help to maintain small spots.

C For qualitative and quantitative work, spot a series of standards representing 0.1 - 1.0 μ g of a pesticide. Tables 1 and 2 present color responses and R_f values for several solvent systems.

VII QUALITY CONTROL

A Duplicate and spiked sample analyses are recommended as quality control

Table 1

R_f Values of O-Aryl Carbamates Pesticides in Several Solvent Systems

	A	B	C	D	E	F
Sevin	0.26	0.22	0.48	0.41	0.58	0.24
Matacil	0.26	0.02	0.46	0.52	0.54	0.04
Zectran	0.34	0.22	0.54	0.53	0.60	0.24
Mesuroi	0.31	0.31	0.55	0.55	0.59	0.28
Baygon	0.27	0.10	0.53	0.59	0.60	0.13

Solvent Systems

- A Hexane/acetone (3:1)
- B Methylene chloride
- C Benzene/acetone (4:1)
- D Benzene/cyclohexane/diethylamine (5:2:2)
- E Ethyl acetate
- F Chloroform

Table 2

Color Responses and Detection Limit for O-Aryl Carbamates

	<u>Colors</u>		<u>Detection Limit (μg)</u>
	<u>Before Buffer</u>	<u>After Buffer</u>	
Sevin	Brown	Red-Purple	0.1
Matacil	Gray	Green	0.1
Zectran	Gray	Green	0.1
Mesuroi	Brown	Tan	0.2
Baygon	Blue	Blue	0.1

checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.

VIII SAMPLE PREPARATION

- A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.
- B Quantitatively transfer a one-liter aliquot into a two-liter separatory funnel.

IX EXTRACTION

- A Add 60 ml methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.
- B Allow the solvent to separate from the sample, draw the organic layer into a 100 ml beaker, then pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Add a second 60 ml volume of solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.
- C Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (X below).
- D If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by thin-layer chromatography (Section 11).

X FLORISIL COLUMN CLEANUP

- A Adjust the sample extract to 10 ml with hexane.

B Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chroma-flex chromatographic column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top.

C Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and separately collect the eluates in 500 ml K-D flasks equipped with 10 ml ampuls. Perform the first elution with 200 ml of 6% ethyl petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether in petroleum ether and the fourth elution with 200 ml of 100% ethyl ether. Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

50% Eluate	100% Eluate
Sevin (70%)	Sevin (30%)
Zectran	Baygon
	Matacil

D Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5 - 1.0 ml.

E Analyze according to XI below.

XI SEPARATION AND DETECTION

A Carefully spot 10% of the extract on a thin-layer. On the same plate spot several pesticides or mixtures for screening purposes or a series of 1, 2, 4, 6, 8 and 10 µl of specific standards for quantitative analysis.

- B Develop the layers 10 cm in a tank saturated with solvent vapors. Remove the plate and allow it to dry.
- C Spray the layer rapidly and evenly with about 10-15 ml chromogenic reagent. Heat the layer in an oven at 110 C for 15 minutes. The pesticides will appear with colors as indicated in Table 2. Make quantitative estimates by visually comparing the intensity and size of the spots with those of the series of standard.
- D Spray the layer with sodium borate reagent and observe the color shift of the reaction products. The color shift must be the same for sample and standard for identification to be confirmed.

XII CALCULATION OF RESULTS

- A Determine the concentration of pesticide in a sample by comparing the response in a sample to that of a quantity of standard treated on the same layer. Divide the result, in micrograms, by the fraction of extract spotted to convert to micrograms per liter

XIII REPORTING RESULTS

- A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

REFERENCES

- 1 "Handbook for Analytical Quality Control in Water and Wastewater Laboratories", Chapter 6, (Section 6.4), U.S. Environmental Protection Agency, National Environmental Research Center, Analytical Quality Control Laboratory, Cincinnati, Ohio 45268, 1972.
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Descriptors: Chemical Analysis Pesticides, Chromatography, Gas Chromatography, Carbamate Pesticides

APPENDIX I

34-7

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XIV STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution (6) (8). An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

- 1 Buret. - 25 ml with 1/10 ml graduations.
- 2 Erlenmeyer flasks. - 125 ml narrow mouth and 25 ml, glass stoppered.
- 3 Pipet. - 10 and 20 ml transfer.
- 4 Volumetric flasks. - 500 ml.

C Reagents and Solvents

- 1 Alcohol, ethyl. - USP or absolute, neutralized to phenolphthalein.
- 2 Hexane. - Distilled from all glass apparatus.
- 3 Lauric acid - Purified, CP.
- 4 Lauric acid solution. - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 5 Phenolphthalein Indicator. - Dissolve 1 g in alcohol and dilute to 100 ml.
- 6 Sodium hydroxide. - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N). Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator;

titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

- 1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130°C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.
- 2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

- 1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = mg lauric acid/g Florisil = $200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05\text{N NaOH})$.

- 2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by (13.6).

F Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and Malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

METHOD FOR N-ARYL CARBAMATE AND UREA PESTICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

- A This method covers the determination of various N-aryl carbamate and urea pesticides in industrial effluents. Such compounds are characterized by the carbamate and urea structures with a nitrogen atom attached to an aromatic ring.
- B The following compounds may be determined individually by this method with a sensitivity of 1 µg/liter: barban, chloropropham, diuron, fenuron, linuron, monuron, neburon, propham, biduron, Swep, Urab and Urox. The usefulness of the method for other specific pesticides must be demonstrated by the analyst before any attempt is made to apply it to sample analysis.
- C The method also detects anilines and can be extended to the detection of anilinic hydrolysis products of the compounds above.

II SUMMARY

- A A measured volume of water is extracted with methylene chloride and the concentrated extract is cleaned up with a Florisil column. Appropriate fractions from the column are concentrated and portions are separated by thin-layer chromatography. The pesticides are hydrolyzed to primary amines, which in turn are chemically converted to diazonium salts. The layer is sprayed with 1-naphthol and the products appear as colored spots. Quantitative measurement is achieved by visually comparing the responses of sample extracts to the responses of standards on the same thin layer. Results are reported in micrograms per liter.
- B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

- A Direct interferences may be encountered from aromatic amines that may be present in the sample. These materials react with the chromogenic reagent and yield reaction products similar to those of the pesticides. In cases where amines are suspected of interfering with a determination, a different solvent system should be used to attempt to isolate the pesticides on the layer.
- B Indirect interferences may be encountered from naturally colored materials whose presence masks the chromogenic reaction.

IV APPARATUS AND MATERIALS

- A Thin-layer plates - Glass plates (200 x 200 mm) coated with 0.25 mm layer of Silica Gel G (gypsum binder).
- B Spotting Template
- C Developing Chamber
- D Sprayer - 20 ml capacity
- E Kuderna-Danish (K-D) Glassware (Kontes)
 - 1 Snyder Column - three ball (K-503000)
 - 2 Micro-Snyder Column - two ball (K-569001)
 - 3 Evaporative Flasks - 500 ml (K-570001)
 - 4 Receiver Amputs - 10 ml graduated (K-570050)
- F Chromatographic Column - Chromaflex (400 mm long x 19 mm ID) with coarse fritted plate on bottom and Teflon stop-cock; 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb - a special order (Kontes K-420540-9011).

G Chromatographic Column - Pyrex (approximately 400 mm long x 20 mm ID) with coarse fritted plate on bottom.

H Micro Syringes - 10, 25, 50 and 100 μ l.

I Separatory Funnel - 2000 ml, with Teflon stopcock.

J Blender - High speed, glass or stainless steel cup.

K Florisil - PR Grade (60-80 mesh); purchase activated at 1250 F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use activate each batch overnight at 130 C in foil-covered glass container. Determine lauric acid value (See Appendix I).

V REAGENTS, SOLVENTS AND STANDARDS

A Ferrous Sulfate - (ACS) 30% solution in distilled water.

B Potassium Iodide - (ACS) 10% solution in distilled water.

C Sodium Hydroxide - (ACS) 10 N in distilled water.

D Sodium Sulfate - (ACS) Granular, anhydrous.

E Sulfuric Acid - (ACS) Mix equal volumes of conc. H_2SO_4 with distilled water.

F Diethyl Ether - Nanograde, redistilled in glass, if necessary.

1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 1% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.

2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate solution to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.

3 Distill deperoxidized ether in glass and add 2% ethanol.

G Hexane, Methanol, Methylene Chloride, Petroleum Ether - nanograde, redistill in glass if necessary

H Pesticide Standards - Reference grade.

I TLC Standards - 0.100 μ g/ μ l in chloroform.

I Nitrous acid - prepare just before use by mixing 1 g $NaNO_2$ with 20 ml 0.2 N HCl.

J Chromogenic agent - Dissolve 1.0 g 1-Naphthol in 20 ml ethanol. Prepare fresh daily.

VI CALIBRATION

A To insure even solvent travel up the layer, the tank used for layer development must be thoroughly saturated with developing solvent before it is used. This may be achieved by lining the inner walls of the tank with chromatography paper and introducing the solvent 1-2 hours before use.

B Samples and standards should be introduced to the layer using a syringe, micropipet or other suitable device that permits all the spots to be about the same size and as small as possible. An air stream directed on the layer during spotting will speed solvent evaporation and help to maintain small spots.

C For qualitative and quantitative work, spot a series representing 0.1-1.0 μ g of a pesticide. Tables 1 and 2 present color responses and R_f values for several solvent systems.

VII QUALITY CONTROL

A Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed, the use of quality control charts is recommended.

VIII SAMPLE PREPARATION

- A Blend the sample if suspended matter is present and adjust pH to near neutral (pH 6.5-7.5) with 50% sulfuric acid or 10 N sodium hydroxide.
- B Quantitatively transfer a one-liter aliquot into a two-liter separatory funnel.

IX EXTRACTION

- A Add 60 ml of methylene chloride to the sample in the separatory funnel and shake vigorously for two minutes.
- B Allow the solvent to separate from the sample, draw the organic layer into a 100 ml beaker, then pass the organic layer through a chromatographic column containing 3-4 inches anhydrous sodium sulfate, and collect it in a 500 ml K-D flask equipped with a 10 ml ampul. Add a second 60 ml volume of solvent to the separatory funnel and complete the extraction procedure a second time. Perform a third extraction in the same manner.
- C Concentrate the extract to 10 ml in a K-D evaporator on a hot water bath. Disconnect the Snyder column just long enough to add 10 ml hexane to the K-D flask and then continue the concentration to about 5-6 ml. If the need for cleanup is indicated, continue to Florisil Column Cleanup (X below).
- D If further cleanup is not required, replace the Snyder column and flask with a micro-Snyder column and continue the concentration to 0.5-1.0 ml. Analyze this final concentrate by thin-layer chromatography (Section XI).

X FLORISIL COLUMN CLEANUP

- A Adjust the Sample Extract to 10 ml With Hexane
- B Place a charge of activated Florisil (weight determined by lauric acid value, see Appendix I) in a Chromaflex chromatographic column. After settling the Florisil by tapping the column,

add about one-half inch layer of anhydrous granular sodium sulfate to the top.

- C Pre-elute the column, after cooling, with 50-60 ml of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Adjust the elution rate to about 5 ml per minute and separately collect the eluates in 500 ml K-D flasks equipped with 10 ml ampuls. Perform the first elution with 200 ml of 6% ethyl ether in petroleum ether, and the second elution with 200 ml of 15% ethyl ether in petroleum ether. Perform the third elution with 200 ml of 50% ethyl ether - petroleum ether and the fourth elution with 200 ml of 100% ethyl ether.

Eluate Composition - By using an equivalent quantity of any batch of Florisil as determined by its lauric acid value, the pesticides will be separated into the eluates indicated below:

15% Eluate	50% Eluate	100% Eluate
CIPC	Barban (5%)	Neburon (92%)
IPC	Linuron	Diuron
Barban (95%)	Neburon (8%)	Fluometuron
		Monuron
		Siduron
		Urox (25%)

CAUTION: Fenuron and Urab are not recovered from the Florisil column. The recovery of Urox is very poor.

- D Concentrate the eluates to 6-10 ml in the K-D evaporator in a hot water bath. Change to the micro-Snyder column and continue concentration to 0.5-1.0 µl.
- E Analyze according to XI below.

XI SEPARATION AND DETECTION

- A Carefully spot 10% of the extract on a thin layer. On the same plate spot several pesticides or mixtures for screening purposes, or a series of 1, 2, 4, 6, 8 and 10 µl of specific standards for quantitative analysis.

B Develop the layers 10 cm in a tank saturated with solvent vapors. Remove the plate and allow it to dry.

C Spray the layer rapidly and evenly with about 10-15 ml sulfuric acid solution. Heat the layer in an oven at 110 C for 15 minutes.

D When the layer is cool, spray it with nitrous acid reagent and allow it to dry. Spray the layer with 1-naphthol reagent and allow it to dry again. The pesticides will appear as purple spots (see Table 2). Identifications are made by comparison of colors and R_f values. Quantitative estimates are made by visually comparing the intensity and size of the spots with those of the series of standard.

XII CALCULATION OF RESULTS

A Determination of concentration of pesticides in a sample by comparing the response in a sample to that of a quantity of standard treated on the same layer. Divide the result, in micrograms, by the fraction of extract spotted to convert to micrograms per liter.

XIII REPORTING RESULTS

A Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed all data obtained should be reported.

Table 1
R_f VALUES OF N-ARYL CARBAMATE AND UREA PESTICIDES
IN SEVERAL SOLVENT SYSTEMS

Carbamates	A	B	C	D	E	F	G
Propham	0.49	0.54	0.73	0.48	0.36	0.68	0.69
Chlorpropham	0.57	0.60	0.73	0.49	0.37	0.70	0.73
Barban	0.61	0.59	0.72	0.41	0.28	0.70	0.74
Swep	0.48	0.44	0.70	0.41	0.28	0.67	0.66
<u>Urea</u>							
Fenuron	0.03	0.04	0.38	0.22	0.10	0.41	0.30
Urab	0.03	0.04	0.36	0.22	0.10	0.41	0.30
Monuron	0.04	0.05	0.37	0.24	0.10	0.47	0.34
Urox	0.04	0.06	0.34	0.24	0.10	0.46	0.34
Diuron	0.05	0.09	0.38	0.28	0.13	0.54	0.44
Linuron	0.40	0.43	0.62	0.39	0.24	0.66	0.64
Neburon	0.21	0.28	0.64	0.41	0.26	0.68	0.65
Siduron	0.02	0.07	0.68	0.39	0.25	0.62	0.55

Solvent Systems

- A Methylene chloride
- B Chloroform
- C Ethyl Acetate
- D Hexane/acetone (2:1)
- E Hexane/acetone (4:1)
- F Chloroform/acetonitrile (2:1)
- G Chloroform/acetonitrile (5:1)

Table 2
COLOR RESPONSES AND DETECTION LIMIT FOR THE
N-ARYL CARBAMATES AND UREAS

<u>Carbamates</u>	<u>Color</u>	<u>Detection Limit (μg)</u>
Propham	Red-purple	0.2
Chlorpropham	Purple	0.1
Barban	Purple	0.05
Swep	Blue-purple	0.2
<u>Ureas</u>		
Fenuron	Red-purple	0.05
Urab	Red-purple	0.1
Monuron	Pink-orange	0.05
Urox	Pink-orange	0.1
Diuron	Blue-purple	0.1
Linuron	Blue-purple	0.1
Neburon	Blue-purple	0.1
Siduron	Red-purple	0.05

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Descriptors: Chemical Analysis Pesticides, Chromatography, Gas Chromatography, Carbamate Pesticides, Urea Pesticides

APPENDIX I

35-9

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XIV STANDARDIZATION OF FLORISIL COLUMN BY WEIGHT ADJUSTMENT BASED ON ADSORPTION OF LAURIC ACID

A A rapid method for determining adsorptive capacity of Florisil is based on adsorption of lauric acid from hexane solution.^{(6), (8)} An excess of lauric acid is used and amount not adsorbed is measured by alkali titration. Weight of lauric acid adsorbed is used to calculate, by simple proportion, equivalent quantities of Florisil for batches having different adsorptive capacities.

B Apparatus

- 1 Buret. - 25 ml with 1/10 ml graduations.
- 2 Erlenmeyer flasks. - 125 ml narrow mouth and 25 ml, glass stoppered.
- 3 Pipet. - 10 and 20 ml transfer.
- 4 Volumetric flasks. - 500 ml.

C Reagents and Solvents

- 1 Alcohol, ethyl. - USP or absolute, neutralized to phenolphthalein.
- 2 Hexane. - Distilled from all glass apparatus.
- 3 Lauric acid. - Purified, CP.
- 4 Lauric acid solution. - Transfer 10.000 g lauric acid to 500 ml volumetric flask, dissolve in hexane, and dilute to 500 ml (1 ml = 20 mg).
- 5 Phenolphthalein Indicator. - Dissolve 1 g in alcohol and dilute to 100 ml.
- 6 Sodium hydroxide. - Dissolve 20 g NaOH (pellets, reagent grade) in water and dilute to 500 ml (1N).

Dilute 25 ml 1N NaOH to 500 ml with water (0.05N). Standardize as follows: Weigh 100-200 mg lauric acid into 125 ml Erlenmeyer flask. Add 50 ml neutralized ethyl alcohol and 3 drops phenolphthalein indicator; titrate to permanent end point. Calculate mg lauric acid/ml 0.05 N NaOH (about 10 mg/ml).

D Procedure

- 1 Transfer 2.000 g Florisil to 25 ml glass stoppered Erlenmeyer flasks. Cover loosely with aluminum foil and heat overnight at 130° C. Stopper, cool to room temperature, add 20.0 ml lauric acid solution (400 mg), stopper, and shake occasionally for 15 min. Let adsorbent settle and pipet 10.0 ml of supernatant into 125 ml Erlenmeyer flask. Avoid inclusion of any Florisil.

- 2 Add 50 ml neutral alcohol and 3 drops indicator solution; titrate with 0.05N to a permanent end point.

E Calculation of Lauric Acid Value and Adjustment of Column Weight

- 1 Calculate amount of lauric acid adsorbed on Florisil as follows:

Lauric Acid value = $\frac{\text{mg lauric acid/g Florisil} = 200 - (\text{ml required for titration} \times \text{mg lauric acid/ml } 0.05\text{N NaOH})}{\text{ml required for titration}}$

- 2 To obtain an equivalent quantity of any batch of Florisil, divide 110 by lauric acid value for that batch and multiply by 20 g. Verify proper elution of pesticides by 13.6.

F Test for Proper Elution Pattern and Recovery of Pesticides: Prepare a test mixture containing aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, Parathion and malathion. Dieldrin and Parathion should elute in the 15% eluate; all but a trace of malathion in the 50% eluate and the others in the 6% eluate.

METHOD FOR CHLORINATED PHENOXY ACID HERBICIDES IN INDUSTRIAL EFFLUENTS

I SCOPE AND APPLICATION

A This method covers the determination of chlorinated phenoxy acid herbicides in industrial effluents. The compounds 2,4-dichlorophenoxyacetic acid (2,4-D), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), 2,3-dichloro-o-anisic acid (dicamba) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) may be determined by this procedure.

B Since these compounds may occur in water in various forms (i.e., acid, salt, ester, etc.) a hydrolysis step is included to permit the determination of the active part of the herbicide. The method may be applied to additional phenoxy acids and certain phenols. However, the analyst must demonstrate the usefulness of the method for each specific compound before applying it to sample analysis.

II SUMMARY

A Chlorinated phenoxy acids and their esters are extracted from the acidified water sample with ethyl ether. The esters are hydrolyzed to acids and extraneous organic material is removed by a solvent wash. The acids are converted to methyl esters which are extracted from the aqueous phase. The extract is cleaned up by passing it through a micro-adsorption column. Identification of the esters is made by selective gas chromatographic separations and may be corroborated through the use of two or more unlike columns. Detection and measurement is accomplished by electron capture, microcoulometric or electrolytic conductivity gas chromatography.⁽¹⁾ Results are reported in micrograms per liter.

B This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons.

III INTERFERENCES

A Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. Refer to Part 1, Sections 1.4 and 1.5.⁽²⁾

B The interferences in industrial effluents are high and varied and often pose great difficulty in obtaining accurate and precise measurement of chlorinated phenoxy acid herbicides. Sample clean-up procedures are generally required and may result in loss of certain of these herbicides. It is not possible to describe procedures for overcoming all of the interferences that may be encountered in industrial effluents.

C Organic acids, especially chlorinated acids, cause the most direct interference with the determination. Phenols including chlorophenols will also interfere with this procedure.

D Alkaline hydrolysis and subsequent extraction eliminates many of the predominant chlorinated insecticides which might otherwise interfere with the test.

E The herbicides, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Glassware and glass wool should be acid-rinsed and sodium sulfate should be acidified with sulfuric acid to avoid this possibility.

IV APPARATUS AND MATERIALS

A Gas Chromatograph - Equipped with glass lined injection port.

B Detector Options:

- 1 Electron Capture - Radioactive (tritium or nickel-63)
- 2 Microcoulometric Titration
- 3 Electrolytic Conductivity

C Recorder - Potentiometric strip chart (10 in.) compatible with the detector.

D Gas Chromatographic Column Materials:

- 1 Tubing - Pyrex (180 cm long x 4 mm ID)
- 2 Glass Wool - Silanized
- 3 Solid Support - Gas-Chrom-Q (100-120 mesh)
- 4 Liquid Phases - Expresses as weight percent coated on solid support.
 - a OV-210, 5%
 - b OV-17, 1.5% plus QF-1, 1.95%

E Kuderna-Danish (K-D) Glassware (Kontes)

- 1 Snyder Column - three ball (macro) and two ball (micro)
- 2 Evaporative Flasks - 250 ml
- 3 Receiver Ampuls - 10 ml, graduated
- 4 Ampul Stoppers

F Blender - High speed, glass or stainless steel cup.

G Graduated cylinders - 100 and 250 ml.

H Erlenmeyer flasks - 125 ml, 250 ml ground glass J 24/40

I Microsyringes - 10, 25, 50 and 100 μ l.

J Pipets - Pasteur, glass disposable (140 mm long x 5 mm ID).

K Separatory Funnels - 60 ml and 2000 ml with Teflon stopcock.

L Glass wool - Filtering grade, acid washed.

M Diazald Kit - recommended for the generation of diazomethane (available from Aldrich Chemical Co., Cat. #210, 025-2)

N Florisil - PR grade (60-100 mesh) purchased activated at 1250F and stored at 130 C.

V REAGENTS, SOLVENTS AND STANDARDS

A Boron Trifluoride-Methanol-esterification-reagent, 14 percent boron trifluoride by weight.

B N-methyl-N-nitroso-p-toluenesulfonamide (Diazald) - High purity, melting point range 60-62 C. Precursor for the generation of diazomethane (see Appendix I).

C Ferrous Sulfate - (ACS) 30% solution in distilled water.

D Potassium Hydroxide Solution - A 37 percent aqueous solution prepared from reagent grade potassium hydroxide pellets and reagent water.

E Potassium Iodide - (ACS) 10% solution in distilled water.

F Sodium Chloride - (ACS) Saturated solution (pre-rinse NaCl with hexane) in distilled water.

G Sodium Hydroxide - (ACS) 10 N in distilled water.

H Sodium Sulfate, Acidified. - (ACS) granular sodium sulfate, treated as follows: Add 0.1 ml of conc. sulfuric acid to 100 g of sodium sulfate slurried with enough ethyl ether to just cover the solid. Remove the ether with the vacuum. Mix 1 g of the resulting solid with 5 ml of reagent water and ensure the mixture to have a pH below 4. Store at 130 C.

I Sulfuric acid. - (ACS) concentrated, Sp. Gr. 1.84.

1 Carbitol (diethylene glycol monoethyl ether).

J Diethyl Ether - Nanograde, redistilled in glass, if necessary.

- 1 Must contain 2% alcohol and be free of peroxides by following test: To 10 ml of ether in glass-stoppered cylinder previously rinsed with ether, add one ml of freshly prepared 10% KI solution. Shake and let stand one minute. No yellow color should be observed in either layer.
- 2 Decompose ether peroxides by adding 40 g of 30% ferrous sulfate to each liter of solvent. CAUTION: Reaction may be vigorous if the solvent contains a high concentration of peroxides.
- 3 Distill deperoxidized ether in glass and add 2% ethanol.

K Benzene Hexane - Nanograde, redistilled in glass, if necessary.

L Pesticide Standards - Acids and Methyl Esters, reference grade.

- 1 Stock standard solutions - Dissolve 100 mg of each herbicide in 60 ml ethyl ether; then make to 100 ml with redistilled hexane. Solution contains 1 mg/ml.
- 2 Working standard - Pipet 1.0 ml of each stock soln into a single 100 ml volumetric flask. Make to volume with a mixture of ethyl ether and hexane (1:1). Solution contains 10 µg/ml of each standard.
- 3 Standard for Chromatography - (Diazomethane Procedure) Pipet 1.0 ml of the working standard into a glass stoppered test tube and evaporate off the solvent using steam bath. Add 2 ml diazomethane to the residue. Let stand 10 minutes with occasional shaking, then allow the solvent to evaporate spontaneously. Dissolve the residue in 200 µl of hexane for gas chromatography.
- 4 Standard for Chromatography - (Boron Trifluoride Procedure) Pipet 1.0 ml of the working standard into a glass stoppered test tube. Add 0.5 ml of Benzene and evaporate to 0.4 ml using a two-ball Snyder micro-

column and a steam bath. Proceed as in (11.3.1). Esters are then ready for gas chromatography.

VI CALIBRATION

- A Gas chromatographic operating conditions are considered acceptable if the response to dicapthion is at least 50% of full scale when < 0.06 ng is injected for electron capture detection and < 100 ng is injected for microcoulometric or electrolytic conductivity detection. For all quantitative measurements, the detector must be operated within its linear response range and the detector noise level should be less than 2% of full scale.
- B Standards, prepared from methyl esters of phenoxy acid herbicides calculated as the acid equivalent, are injected frequently as a check on the stability of operating conditions.
- C The elution order and retention ratios of methyl esters of chlorinated phenoxy acid herbicides are provided in Table 1, as a guide.

VII QUALITY CONTROL

- A Duplicate and spiked sample analyses are recommended as quality control checks. When the routine occurrence of a pesticide is being observed the use of quality control charts is recommended.⁽³⁾
- B Each time a set of samples is extracted, a method blank is determined on a volume of distilled water equivalent to that used to dilute the sample.

VIII SAMPLE PREPARATION

- A Blend the sample, if suspended matter is present.
- B For a sensitivity requirement of 1 µg/l, when using electron capture for detection, take 100 ml of sample for analysis. For microcoulometric or electrolytic conductivity detection, take 1-liter of sample. Background information on the extent and nature of interferences will assist the analyst in selecting the proper sample size and detector.

Table 1

RETENTION RATIOS FOR METHYL ESTERS OF SOME CHLORINATED
PHENOXY ACID HERBICIDES RELATIVE TO 2,4-D

Liquid Phase ¹	1.5% OV-17 + 1.95% QF-1	5% OV-210
Column Temp.	185 C	185C
Argon/Methane Carrier Flow	70 ml/min	70 ml/min
Herbicide	RR	RR
dicamba	0.60	0.61
2,4-D	1.00	1.00
silvex	1.34	1.22
2,4,5-T	1.72	1.51
2,4-D (minutes absolute)	2.00	1.62

¹ All columns glass, 180 cm x 4 mm ID, solid support
Gas Chrom Q (100/120 mesh)

- C Quantitatively transfer the proper aliquot of sample into a two-liter separatory funnel, dilute to one liter and acidify to approximately pH 2 with concentrated sulfuric acid. Check pH with indicator paper.

IX EXTRACTION

- A Add 150 ml of ether to the sample in the separatory funnel and shake vigorously for one minute.
- B Allow the contents to separate for at least ten minutes. After the layers have separated, drain the water phase into a one-liter Erlenmeyer flask. Then collect the extract in a 250 ml ground-glass Erlenmeyer flask containing 2 ml of 37 percent aqueous potassium hydroxide.
- C Extract the sample two more times using 50 ml of ether each time, and combine the extracts in the Erlenmeyer flask. (Rinse the one-liter flask with each additional aliquot of extracting solvent.)

X HYDROLYSIS

- A Add 15 ml of distilled water and a small boiling stone to the flask containing the ether extract, and fit the flask with a 3-ball Snyder column. Evaporate the ether on a steam bath and continue heating for a total of 60 minutes.
- B Transfer the concentrate to a 60 ml separatory funnel. Extract the basic solution two times with 20 ml of ether and discard the ether layers. The herbicides remain in the aqueous phase.
- C Acidify the contents of the separatory funnel by adding 2 ml of cold (4 C) 25 percent sulfuric acid (5.9). Extract the herbicides once with 20 ml of ether and twice with 10 ml of ether. Collect the extracts in a 125 ml Erlenmeyer flask containing about 0.5 g of acidified anhydrous sodium sulfate (5.8). Allow the extract to remain in contact with the sodium sulfate for approximately two hours.

XI ESTERIFICATION (4, 5)

- A Transfer the ether extract, through a funnel plugged with glass wool, into a Kuderna-Danish flask equipped with a 10 ml graduated ampul. Use liberal washings of ether. Using a glass rod, crush any caked sodium sulfate during the transfer.

- 1 If esterification is to be done with diazomethane, evaporate to approximately 4 ml on a steam bath (do not immerse the ampul in water) and proceed as directed in Section 11. 2
- 2 If esterification is to be done with boron trifluoride, add 0.5 ml benzene and evaporate to about 5 ml on a steam bath. Remove the ampul from the flask and further concentrate the extract to 0.4 ml using a two-ball Snyder microcolumn and proceed as in 11. 3.

B Diazomethane Esterification

- 1 Disconnect the ampul from the K-D flask and place in a hood away from steam bath. Adjust volume to 4 ml with ether, add 2 ml diazomethane, and let stand 10 minutes with occasional swirling.
- 2 Rinse inside wall of ampul with several hundred microliters of ethyl ether. Take sample to approximately 2 ml to remove excess diazomethane by allowing solvent to evaporate spontaneously (room temperature).
- 3 Dissolve residue in 5 ml of hexane. Analyze by gas chromatography.
- 4 If further clean-up of the sample is required, proceed as in 11. 3. 4 substituting hexane for benzene.

C Boron Trifluoride Esterification

- 1 After the benzene solution in the ampul has cooled, add 0.5 ml of borontrifluoride-methanol reagent. Use the two-ball Snyder microcolumn as an air-cooled condenser and hold the contents of the ampul at 50 C for 30 minutes on the steam bath.

2 Cool and add about 4.5 ml of a neutral 5 percent aqueous sodium sulfate solution so that the benzene-water interface is in the neck of the Kuderna-Danish ampul. Seal the flask with a ground glass stopper and shake vigorously for about one minute. Allow to stand for three minutes for phase separation.

3 Pipet the solvent layer from the ampul to the top of a small column prepared by plugging a disposable Pasteur pipet with glass wool and packing with 2.0 cm of sodium sulfate over 1.5 cm of Florisil adsorbent. Collect the eluate in a graduated ampul. Complete the transfer by repeatedly rinsing the ampul with small quantities of benzene and passing the rinses through the column until a final volume of 5.0 ml of eluate is obtained. Analyze by gas chromatography.

XII CALCULATION OF RESULTS

A Determine the methyl ester concentration by using the absolute calibration procedure described below or the relative calibration procedure described in Part I, Section 3.4.2. (2)

$$\text{Micrograms/liter} = \frac{(A) (B) (V_t)}{(V_i) (V_s)}$$

A = $\frac{\text{ng standard}}{\text{Standard area}}$

B = Sample aliquot area

V_i = Volume of extract injected (μ l)

V_t = Volume of total extract (μ l)

V_s = Volume of water extracted (ml)

B Molecular weights for the calculation of the methyl esters as the acid equivalents.

2,4-D	222.0
2,4-D Methyl ester	236.0
Silvex	269.5
Silvex methyl ester	283.5
Dicamba	221.0
Dicamba methyl ester	236.1
2,4,5-T	255.5
2,4,5-T methyl ester	269.5

XIII REPORTING RESULTS

A Report results in micrograms per liter as the acid equivalent without correction for recovering data. When duplicate and spiked samples are analyzed all data obtained should be reported

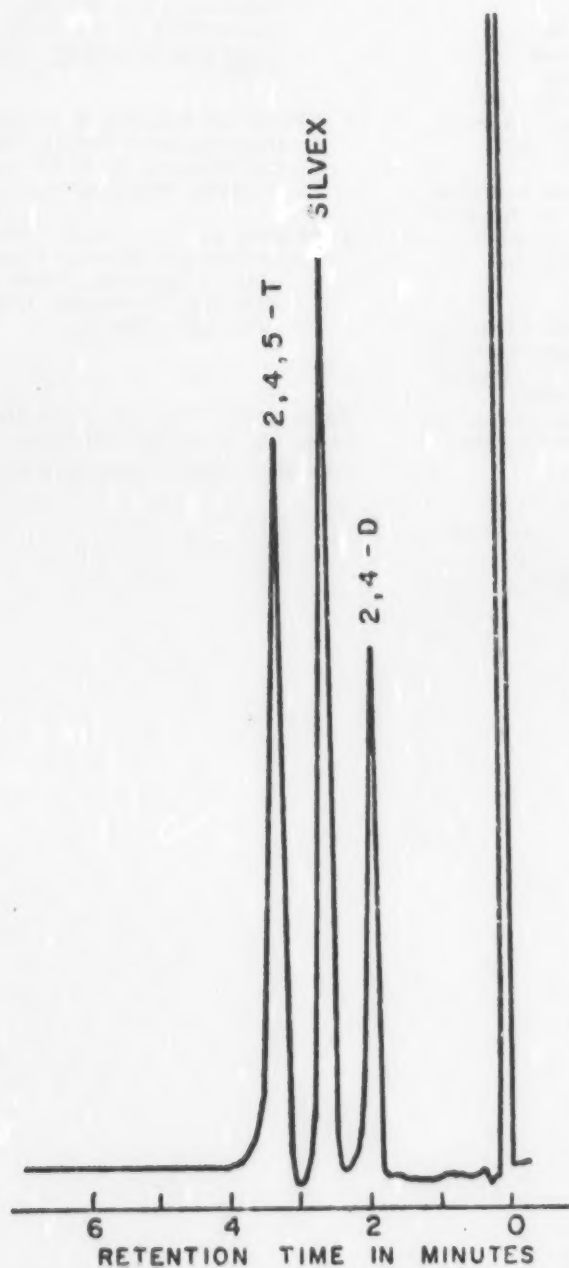


Fig. 1 Column: 1.5 % OV-17 + 1.95 % QF-1,
Carrier Gas: Argon (5%) / Methane: 70ml/min.,
Column Temp. 185 C, Detector: Electron Capture.

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Descriptors: Analytical Techniques, Chemical Analysis Pesticides, Chromatography, Gas Chromatography, Herbicides

APPENDIX I

36-9

Diazomethane in ether.⁽⁶⁾

1. CAUTIONS

Diazomethane is very toxic. It can explode under certain conditions. The following precautions should be observed:

Avoid breathing vapors.

Use only in well-ventilated hood.

Use safety screen.

Do not pipette solution of diazomethane by mouth.

For pouring solutions of diazomethane, use of gloves is optional.

Do not heat solutions to 100 C (EXPLOSIONS).

Store solutions of gas at low temperatures (Freezer compartment of explosion proof refrigerators).

Avoid ground glass apparatus, glass stirrers and sleeve bearings where grinding may occur (EXPLOSIONS).

Keep solutions away from alkali metals (EXPLOSIONS).

Solutions of diazomethane decompose rapidly in presence of solid material such as copper powder, calcium chloride, boiling stones, etc. These solid materials cause solid polymethylene and nitrogen gas to form.

2. PREPARATION

Use a well-ventilated hood and cork stoppers for all connections. Fit a

125 ml long-neck distilling flask with a dropping funnel and an efficient condenser set downward for distillation. Connect the condenser to two receiving flasks in series - a 500 ml Erlenmeyer followed by a 125 ml Erlenmeyer containing 30 ml ether. The inlet to the 125 ml Erlenmeyer should dip below the ether. Cool both receivers to 0 C.

As water bath for the distilling flask, set up a 2-liter beaker on a stirplate (hot plate and stirrer), maintaining temperature at 70 C.

Dissolve 6 g KOH in 10 ml water in the distilling flask (no heat). Add 35 ml Carbitol (diethylene glycol monoethyl ether), stirring bar, and another 10 ml ether. Connect the distilling flask to the condenser and immerse distilling flask in water bath. By means of the dropping funnel, add a solution of 21.5 g Diazald in 140 ml ether over a period of 20 minutes. After distillation is apparently complete, add another 20 ml ether and continue distilling until distillate is colorless. Combine the contents of the two receivers in a glass bottle (WITHOUT ground glass neck), stopper with cork, and freeze overnight. Decant the diazomethane from the ice crystals into a glass bottle, stopper with cork, and store in freezer until ready for use. The final solution may be stored up to six months without marked deterioration.

The 21.5 g of Diazald reacted in this manner produce about 3 g of Diazomethane.

TABLE 1

ORDER OF ELUTION OF STANDARD INSECTICIDES FROM ALUMINA ADSORPTION COLUMN

Insecticide											Weight Insecticide Used μg	% Recovery (Avg. of 3 Runs)
	1	2	3	4	5	6	7	8	9	10		
DDE	95	5									10	94
Aldrin	93	7									10	97
Heptachlor	70	30									10	96
Chlordane (Tech.)	30	30	40	Trace							20	99
Toxaphene	15	55	30	Trace							50	93
DDT	5	95									20	94
γ Chlordane		2	80	18							10	99
DDD			60	40							10	93
Lindane			35	65							10	40
Endrin				45	55						10	95
Hept. Epoxide				35	50	15					10	95
Dieldrin						20	40	20	15	5	10	96
Methoxychlor						5	30	50	10	5	50	96
Lindane	(Acid alumina)				25	60	15				10	100
Lindane	(Neutral alumina)			3	75	20	2				10	91

Eluting Solvent - 9:1 Hexane/Ethyl ether.
 Elution Rate - 3.5 to 4.0 ml per minute.

TABLE 2
PHYSICAL CONSTANTS OF SOLVENTS

Solvent	Dielectric Constant (Polarity)	b. p. °C	Density g/ml	*Sol. at R. T. in H ₂ O
Pentane	1.84	36	0.626	Slightly soluble
Hexane	1.89	69	0.660	Insoluble
Isooctane				
(2, 2, 4-Trimethylpentane)	1.94	99	0.692	Insoluble
Cyclohexane	2.02	81	0.779	Insoluble
1, 4-Dioxane	2.21	101	1.03	Miscible
Carbon Tetrachloride	2.24	77	1.59	Insoluble
Benzene	2.28	80	0.879	Slightly soluble
Toluene	2.38	111	0.867	Insoluble
Carbon Disulfide	2.64	46	1.26	Soluble
Ethyl Ether	4.34	35	0.714	Slightly soluble
Chloroform	4.81	62	1.48	Slightly soluble
Ethyl Acetate	6.02	77	0.900	Soluble
Methylene Chloride	9.08	40	1.33	Slightly soluble
t-Butanol	10.9	82	0.789	Miscible
Pyridine	12.3	116	0.982	Miscible
1-Butanol	17.1	117	0.810	Soluble
2-Propanol	18.3	82	0.786	Miscible
2-Butanone (MEK)	18.5	80	0.805	Very soluble
1-Propanol	20.1	97	0.804	Miscible
Acetone	20.7	56	0.790	Miscible
Ethanol	24.3	79	0.789	Miscible
Methanol	32.8	65	0.791	Miscible
Acetonitrile	37.5	82	0.786	Miscible
N, N-Dimethylformamide	37.6	149	0.943	Miscible
Dimethyl Sulfoxide	46.7	189	1.10	Very soluble
Water	78.5	100	1	

* Insoluble < Slightly soluble < Soluble < Very soluble < Miscible